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WILLOW INNER BARK AS A POTENTIAL SOURCE OF FIBRES AND CHEMICALS

Master's thesis for the degree of Master of Science in Technology submitted for inspection, Espoo, 19.05.2015

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Abstract of Master's Thesis

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Abstract

The aim of this thesis was to acquire basic information on the physical and chemical structure of willow inner bark in order to assess its potential as a raw material for chemicals and fibres. Inner bark from four cultivated willow species/hybrids was studied and compared with their wood tissue. The cell and cell wall structure was studied by optical microscopy, SEM and TEM. The fibres were separated with an acid chlorite treatment and analyzed for their dimensions and morphology. The chemical composition was determined by standard methods including quantification of ash, extractives (extraction with acetone), lignin and carbohydrates. FTIR spectroscopy and Raman spectroscopy were applied as additional characterization methods.

In comparison with willow wood fibres the sclerenchyma fibres of inner bark were much longer and they had higher aspect ratio. The inner bark fibres were thick-walled and their lumina were almost nonexistent. The inner bark had almost ten times as high ash and extractives contents as the willow wood had. The lignin-to-polysaccharide ratio was similar in wood and bark although pectin/arabinogalactan was the dominant heteropolysaccharide in inner bark over xylan in wood.

The excellent fibre properties and the high extractives content of inner bark may justify its separation from willow biomass for production of high-value special fibres and specific extractive-based chemical compounds. The debarked biomass could be used for production of sugars, lignin and their derivatives.

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Jinze Dou

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LIST OF ABBREVIATIONS

DP	Degree of polymerization
L/W	Liquor to wood ratio
ASL	Acid soluble lignin
TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
RM	Raman micro-spectroscopy
FTIR	Fourier transform infrared (spectroscopy)
HPAEC	High performance anion exchange chromatography
UV	Ultraviolet
UVRR	UV resonance Raman (spectroscopy)
DWL	Dissolved wood lignin
ASL	Acid soluble lignin
CHP	Combined heat and power
PEG	Polyethylene glycol

Contents

1 Introduction

1.1 Background

With increasing industrialization, fossil fuels (coal, oil and natural gas) have become the main source for energy production but their use has increased carbon dioxide emissions with the consequent influence on the phenomenon of global warming. For this reason, under the concept of bio-refinery, the development of bio-renewable chemicals and fuels from non-food plant materials constitute an important task to undertake not only to solve escalating demand of energy and chemicals but also to reduce the damage caused by the global climate. One promising raw material for the production of such fuels and chemicals is lignocellulose biomass (vom Stein, Grande et al. 2011).

Lignocellulose biomass is formed by a reduction-oxidation reaction between carbon dioxide and water with the sunlight at chlorophyll in plants. It consists of cross-linked polysaccharide (cellulose, hemicellulose) networks and lignin, as well as small amounts of extractives and inorganics. Lignocellulose biomass has been long recognized as a potential source of sugars and ethanol derived from them by fermentation (Sukumaran, Singhania et al. 2009). In this regard the annual plants as willow (genus Salix) with higher productivity and the short rotation coppice production than normal woody biomass might be adopted as sustainable biomass production to generate bioenergy in an environmental sustainable way (Volk, Abrahamson et al. 2006).

Willow is a highly diversified genus of trees, consisting of more than 350-400 species. Willow has been studied as energy crop, based on the benefits of high productivity and strong adaptability as well as the capability to grow from the peat lands that after peat harvesting. The total forestry land area in Finland is 26 million ha, of which 6 million ha is of low productivity (< 1.0 m^3/ha/a). Willow growth on forestry land of low productivity can be up to 20 times (6-8 dry tons/ha/a) higher than wood growth on forestry land of low productivity (Toivonen, Tahvanainen 1998). Although different parts of willow trees such as bark and sap have been used in traditional applications as fibre source for handicraft and medicine, willow bark (inner and outer) is generally being considered a waste product that must be removed in order to obtain the desired wood products and burned off for producing electricity and heat or used for landfill.

Despite the great potential of the willow inner bark as fibre source and raw material in the chemical industry, most of studies for willow have focused on the cultivation of willow for combined heat and power (CHP) generation (Keoleian, Volk 2005), however, the high contents of water and bark in willow biomass have been considered to be challenges for CHP production. Moreover, very little is known about the chemical composition and morphology of willow fractions, or about the structure of willow lignin. It is believed that willow biomass could be used more innovatively, especially throughout fractionation into bark and sapwood. Several studies on bark composition have been performed in other species like Norway spruce to evaluate hydrophilic extractive content and tannins as well as Pakistani coniferous tree species to analyse the lipophilic extractive content (Krogell 2012; Kubo, Hashida et al. 2013; Sen, Miranda et al. 2010). Preliminary experiments made in our department with willow inner bark have already revealed that normal Kraft cooking is able to produce good quality fibres and unique sclerenchyma fibre bundles, superior to any known softwood and hardwood fibres for paper, packaging and composites application.

In the present work, structural and chemical characterisation of four different clones of willow inner bark available in Finland was carried out as first step in studying the implementation of a bio-refinery concept for willow species. To do so, firstly microstructural characterizations of willow inner bark by using optical microscopy and SEM (Scanning electron microscopy) as well as TEM (Transmission electron microscopy) were performed. Secondly, physical properties of fibres from willow inner bark (length and aspect ratio) were

evaluated. Finally, the chemical composition of inner bark namely lignin, extractives and sugars were also determined.

1.2 Research objectives

The aim of this work is to perform structural and chemical characterization of the willow inner bark of four different clones for its use as potential raw material for bio-based products. Several partial objectives were defined in order to accomplish the main objective:

- To characterize native willow inner bark by using several microscopy techniques such as optical microscopy, Transmission Electron Microscopy (TEM) and Scanning Electron Microscope (SEM**).** Raman micro-spectroscopy (RM) and UV Resonance Raman spectroscopy (UVRR).
- To evaluate physical properties of willow inner bark fibres.
- To determine the overall chemical composition of willow inner bark namely lignin, extractives and sugars.

2 Literature review

2.1 Wood structure

2.1.1 Cellulose

The cellulose molecule is built up by repeating cellobiose residues which are formed by two adjacent glucose units attached by replacing one water molecule, thus producing anhydroglucose units. Cellulose constitutes the major part of the wood section by holding 40-45% of the dry matter in most of the wood species. Cellulose is a structural polysaccharide and the most abundant natural polymer in the world, which is a linear homopolymer composed of D-anhydroglucose units that are linked by β-(1, 4)-glycosidic bonds. Generally, three hydroxyl groups exist in every anhydroglucose units (GOLDSTEIN 1983). The molecular structure of cellulose is shown in Figure 1.

Due to the high degree of crystallization (9000-15000 glucose units), only few solvents can dissolve cellulose, a number of methods can be listed here, which are visco method, Lyocell, ionic liquid, and alkaline mixture in water (Hult 2001; GARDNER, BLACKWEL.J 1974).

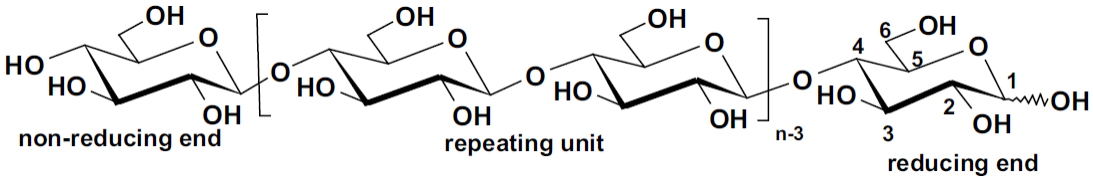

Figure 1. The structure of cellulose.

2.1.2 Hemicellulose

Wood contains other polysaccharides besides cellulose called hemicelluloses. These are heterogeneous polysaccharides with lower degree of polymerization (100-200 glucose units) compared to cellulose, with various side groups, substituents, depending on the wood species. Generally, galactoglucomannans and 4-O-methyl-glucuronoxylans are the main hemicelluloses in softwood and hardwood respectively. However, the hemicelluloses are mainly composed of glucuronoarabinoxylans in the herbaceous grass (Jørgensen, Kristensen et al. 2007). Figures 2 and 3 show the chemical structure of xylans (Schild, Sixta et al. 2010; GOLDSTEIN 1983).

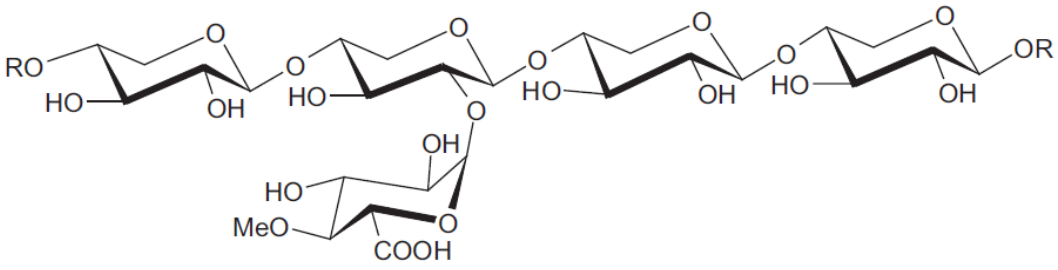

Figure 2. O-acetyl-4-O-methylglucuronoxylan (glucoronoxylan).

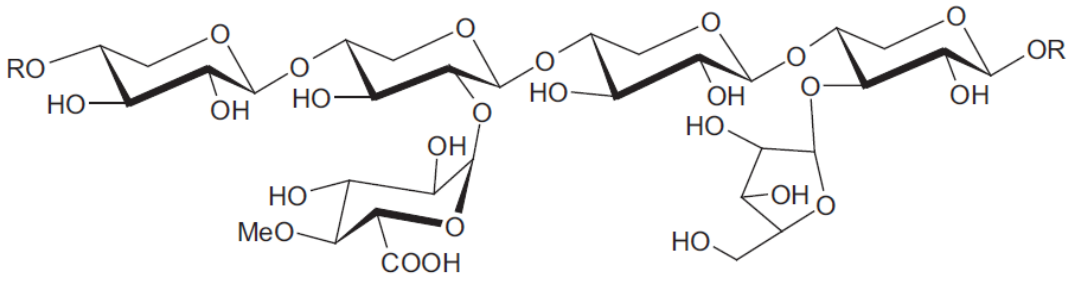

Figure 3. Arabinoglucuronoxylan.

2.1.3 Lignin

Lignin is a cross-linked amorphous polymer, which contains three important basic units: p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) (Leschinsky, Zuckerstaetter et al. 2008) (Figure 4). Guaiacyl unit is the main constituent in softwood lignin, while guaiacyl and syringyl are commonly found in hardwood. Furthermore, minor amount of *p*-hydroxyphenyl units can be found in both of softwood and hardwood lignin (Jørgensen, Kristensen et al. 2007; Zhao, Zhang et al. 2012).

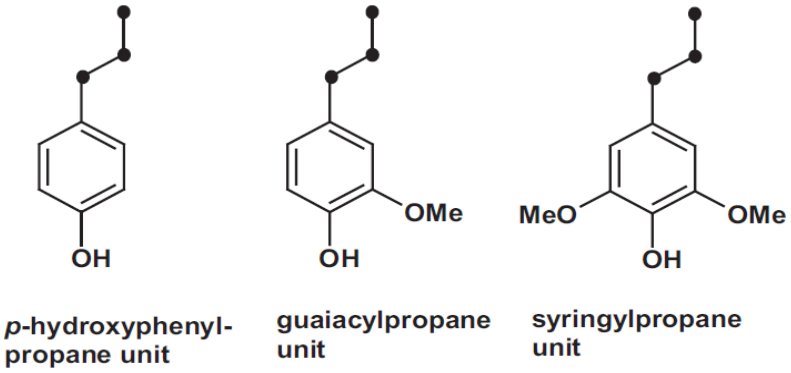

Figure 4. Structure of lignin precursors.

Lignin has been described as a random, three-dimensional network polymer comprised of variously linked phenylpropane units. The most predominant functional groups in lignin are hydroxyl, methoxyl, carbonyl and carboxylic acid groups. From the Figure 5, it is possible to observe that these units are joined together by ether linkages (C-O-C) and carbon-carbon bonds (C-C), the most prominent linkage type in wood is the β-O-4 structure (Vanholme, Demedts et al. 2010).

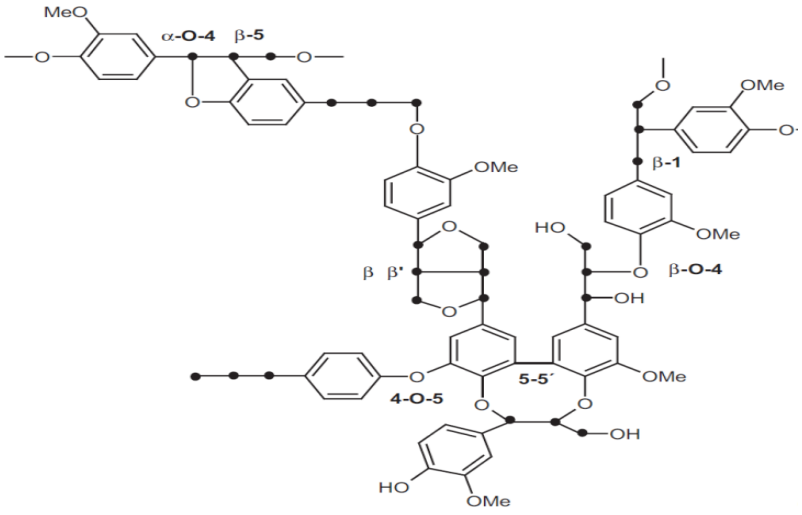

Figure 5. Structure of lignin segment and its main linkages.

2.2 Bark structure

2.2.1 Anatomy of bark

Bark is a non-technical word that has been used to represent the outermost covering layer of tree stems, which is more complex in comparison to wood section (MCDONOUGH 1983). According to (Harkin 1971), after wood the bark is the second most important tissue of a tree trunk and it is formed by a process of cell division at the vascular cambium layer.

Generally, the bark is composed of the living inner bark or phloem and the outer bark or rhytidome. The outer bark is being formed by the squeezing and extruding of the outer layer of the phloem during the primary or secondary growth. Continuous division during the growth period promotes the growth of the periderm, which is the boundary between the inner bark and outer bark tissues (Sandved et al. 1992, p. 24).

2.2.1.1 Inner bark

Inner bark is produced by and adjacent to the vascular cambium (Figure 6) and is composed of cells (parenchyma and sclerenchyma cells) and the sieve elements (Sandved et al. 1992, p. 25). The sieve elements can be segmented into sieve cells and the sieve tubes in gymnosperms and angiosperms respectively, where they distributes through the sieve areas that being arranged in longitudinal cell rows for the transportation of the liquids and nutrients. When inner bark develops towards the outside of the stem, so called periderm is produced by the phellogen. The phellogen develops from parenchyma cells of the older phloem tissue (MCDONOUGH 1983).

Sclerenchyma cells that serve the function of support in plants are dead cells that have heavily thickened walls that contain lignin. Such cells occur in many different shapes and sizes, but two main types occur: fibres and sclereids. The fibres provide maximum support to the plant, they can be found almost anywhere in the plant body, including the stem, the roots, and the vascular bundles from the bark section. Sclereids are evenly distributed from the periderm and xylem as well as the phloem (Chesson, Provan et al. 1997; MCDONOUGH 1983). It is reported that the thickness of the inner bark remains about constant during growth, so old inner bark is turned into new outer bark at about the same rate as new inner bark forms at the cambium surface (Potgieter 1994).

2.2.1.2 Outer bark

Outer bark, also called rhytidome, includes all of the tissues from the innermost periderm to the outside of the stem, which functions against the mechanical damage and the potential weather variation (MCDONOUGH 1983). The entire outer bark consists of the periderm layers and frequently old dead phloem layers. The phellogen, also called the cork cambium produces cork cells to the outside. These cork cells are usually tightly packed and have fatty substances (waxes and suberin) deposited in their walls. The fatty substances give cork its special property, like the ability to restrict the passage of water. Cork cells are typically dead, and their interiors are filled with air. Hence, cork is usually light in weight and provides thermal insulation. Sometimes they may contain other organic substances, such as tannins or resins which give them colour (Sandved et al. 1992).

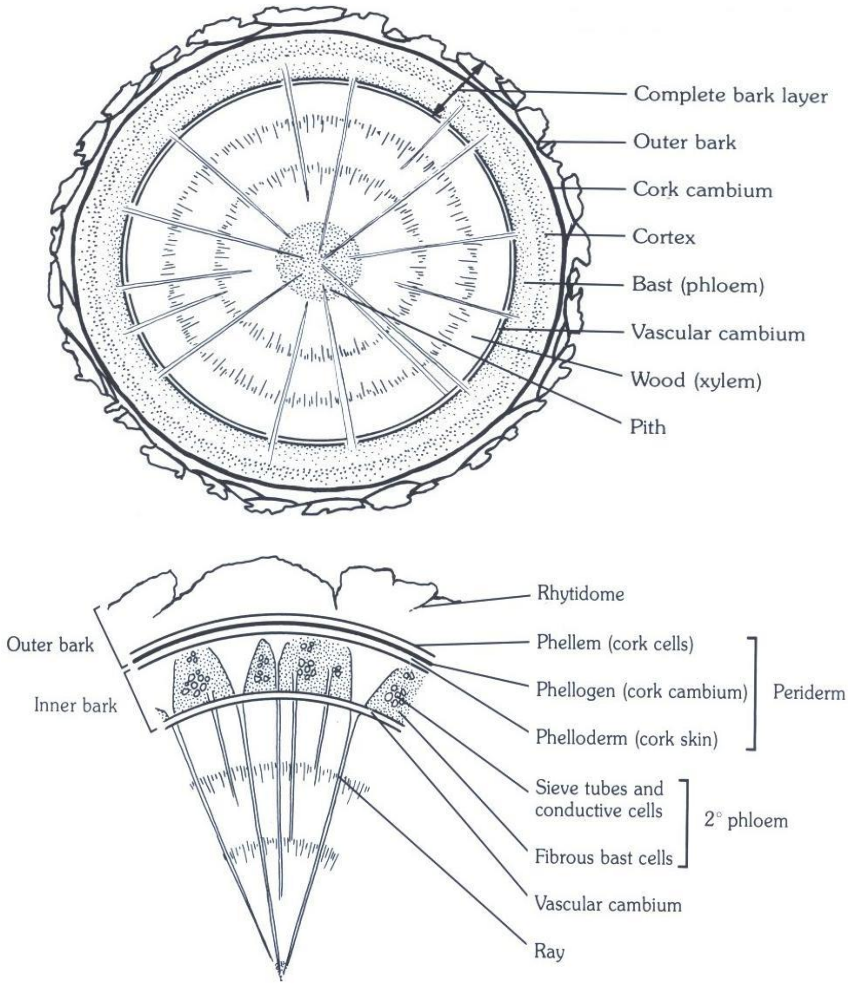

Figure 6. Cross section through the trunk of a three-year old tree showing the bark tissues.

2.2.2 Chemical composition of bark

Components present in wood are often present in bark, although the proportions are different among tree species. Bark is constituted by fibres, cork cells and the fine substance (parenchyma cells). Large quantity of complex chemical compounds can be isolated from bark cork cells, such as flavonoids, alkaloids, carbohydrates, inositols, terpenoids, glycosides, saponins fats and complex phenols. Similarly, the fibre fraction consists of the lignin, cellulose and hemicelluloses (Krogell, Holmbom et al. 2012; Egbung, et al. 2013).

2.2.2.1 Extractives

Bark extractives are much abundant and complex than in wood. Normally, bark extractives (lipophilic and hydrophilic fractions) can account for the 20-40% of the dry weight of bark (MCDONOUGH 1983). Lipophilic components such as fats, waxes, terpenoids and the high sterols are extracted by the nonpolar solvents (ethyl ether, etc.) whereas hydrophilic fraction are extracted by the polar organic solvents (acetone, ethanol) or water alone. The condensed tannins are extracted as salts using dilute solutions of alkali. Overall, the amount of polar components (tannins, polyphenols, and glycosides) are three to five times higher than nonpolar constituents (fats, waxes, terpenes, steroids, etc) (MCDONOUGH 1983).

2.2.2.2 Insoluble constituents

The bark cell wall is composed mainly of the polysaccharides, lignin and the suberins. The cellulose and the hemicelluloses are quite similar to the relevant wood material, as shown by Table 1. For example the

polysaccharide named (1->3)-β-D-glucan distributes from the sieve elements to connect the β-D-glucan endo- and exo-hydrolases in higher plants (Harkin 1971).

Table 1. Proximate composition of ash-free wood and bark (percent).

	Softwoods		Hardwoods	
	wood	**bark**	**wood**	**bark**
Lignin *	25-30	40-55	18-25	40-50
Polysaccharides *	66-72	30-48	74-80	32-45
Extractives	2-9	2-25	2-5	5-10
Ash *	0.2-0.6	up to 20	0.2-0.6	up to 20

* based on extractive free material

The high analysed lignin content in Table 1 indicates that the actual lignin content in the bark is comparatively higher than in the wood section. The difficulty of separating the phenolic acids from the bark makes it difficult to get the satisfactory data for the lignin portions of the whole bark. (MCDONOUGH 1983) also mentioned that the suberin components from the outer bark are as high as 20-40% from the periderm of birch barks (Krogell, Holmbom et al. 2012).

2.2.2.3 Inorganic constituents

The inorganic compounds accounts for 2-5% of the dry bark weight. Calcium and potassium are the main metals present, bound by carboxylate groups. Bark also stores some trace elements, such as the boron, copper and the manganese (MCDONOUGH 1983).

2.3 Bark applications

As Figure 7 shows, bark has a long history as raw material for handicraft. For example in the production of the Laos's rustic wild crafted textile and the modern snowflake of Japanese workshop. Several studies have been done to contribute more added value to bark residues from wood industries. Bark could be used as a source of unconventional chemicals, including the adhesives and the pharmaceutics (Ogunwusi, A.A, 2013).

Figure 7. Wild crafted textile fibres (blanket) from Laos and the Japanese handicraft snowflake made from inner bark (Chrubasik, Kunzel et al. 2001; Jones 2011).

2.3.1 Fuel

Fuel can be regarded as the low-grade utilization of wood bark. Generally, bark has a great burning value as wood, the fuel value for the heartwood and the dry bark yield 21 and 23 $MJkg^{-1}$, respectively. The more resinous the bark, the greater its fuel value is. Thus, the pine bark is a rich source of energy that can become increasingly important as a source of fuel as the petroleum suppliers decline (Sandved et al. 1992, p.125). However, the bark is not recommended as a material for burning since its low calorific value that results from the high moisture content. In addition, the burning equipment needs high investment to control the environmental emissions. Meanwhile, the high bark content also increases the yield of charcoal production, although the charcoal from the bark is more easily crumbled than the wood charcoal and also has high ash content. Thus bark could have higher economical potential for composite board production (Harkin 1971).

2.3.2 Hydrophobic and amphiphilic components

There are several examples on exploitation of the hydrophobic or amphiphilic components of bark. The baobab fibres are woven into water-proof hats that double as drinking vessels from Senegal and Ethiopia; In Peru and Chile, the saponins present in the willow inner bark can also be used as soap and emulsifier. The bark of the soapbox tree (Quillaja saponaria) is used as a substitute for soap; in southern Brazil and eastern Argentina, Quillaja brasiliensis is used as soap. The inner bark is dried and then powdered for the application as an emulsifying agent in tars and hair shampoo as well as lather forming (Sandved et al. 1992, p. 126).

2.3.3 Adhesives

The green adhesives and the bark-based foams mitigate the climatic change by the potential of replacing the petroleum-based products, the idea of using tannins as a substitute to phenols from the bark for adhesive application can make the higher economic and environmental benefits (Ogunwusi, A.A, 2013). The idea of using the tannin from the bark for adhesive application has already been reported by many authors (ZHAO, CAO et al. 1994; YAZAKI, COLLINS 1994). Tannin also being defined as the phenolic compound of high molecular weight that containing sufficient hydroxyls and other carboxyl groups that can form effectively strong complexes with protein, which make it possible to modify the animal skins and hides into leather by associating the hydroxyl group (tannin) and the peptide bonds of the amino acids that present in the animal proteins (collagen) (Ogunwusi, A.A, 2013).

2.3.4 Medicine

Hokkanen (2012) reported that the application of synthetic derivatives and analogous would be the most promising field for antimicrobials, the ForestSpeCs project checked out one derivatives of birch bark terpene called betulin that restrain the growth of parasites from the tropical disease, additionally, the extremely hydrophobic property of betulin shows the potential application in the cosmetic area.

2.4 Analytical techniques

2.4.1 Raman spectroscopy

Raman spectroscopy is a technique that can measure samples with minimal preparation (gas, liquid, solid) based on inelastic scattering of a monochromatic excitation source - Raman scattering, when a molecule is subjected to monochromatic light (laser), usually from a laser source. Photons of the laser light are absorbed by the sample and then remitted. The electrons and nuclei are forced to shift in opposite directions for the comparison with original monochromatic frequency. Then the shift provides information about vibrational, rotational and other low frequency transitions in molecules (Lähdetie 2013).

Wood is composed of lignin (20-30%), hemicellulose (25-30%) and cellulose (40%), and the distribution of components vary greatly within wood cell walls, commonly only fractionated samples have been measured (isolated samples). However, the determination of characteristic Raman bands for a single compound is very difficult, that is because of the presence of multiple compounds with overlapping bands that make the detection difficult (Lähdetie 2013). In nature, cellulose is found associated in micro fibrils and the micro fibrils are oriented in different directions in different cell walls, like Figure 9 illustrates (Zakaznova-Herzog, Malfait et al. 2007). According to Gierlinger (2010), Raman band arising from glycoside bond of cellulose can be used to detect the orientation of the willow's fibre, as Figure 8 shows to us.

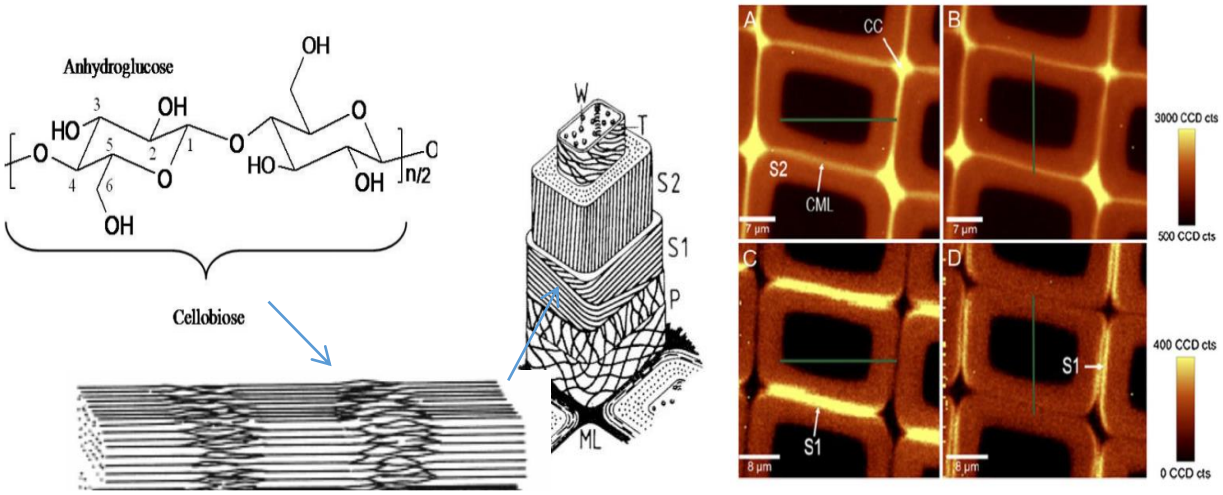

Figure 8. Cellulose structure and Raman mapping of lignin (above) and cellulose (below).

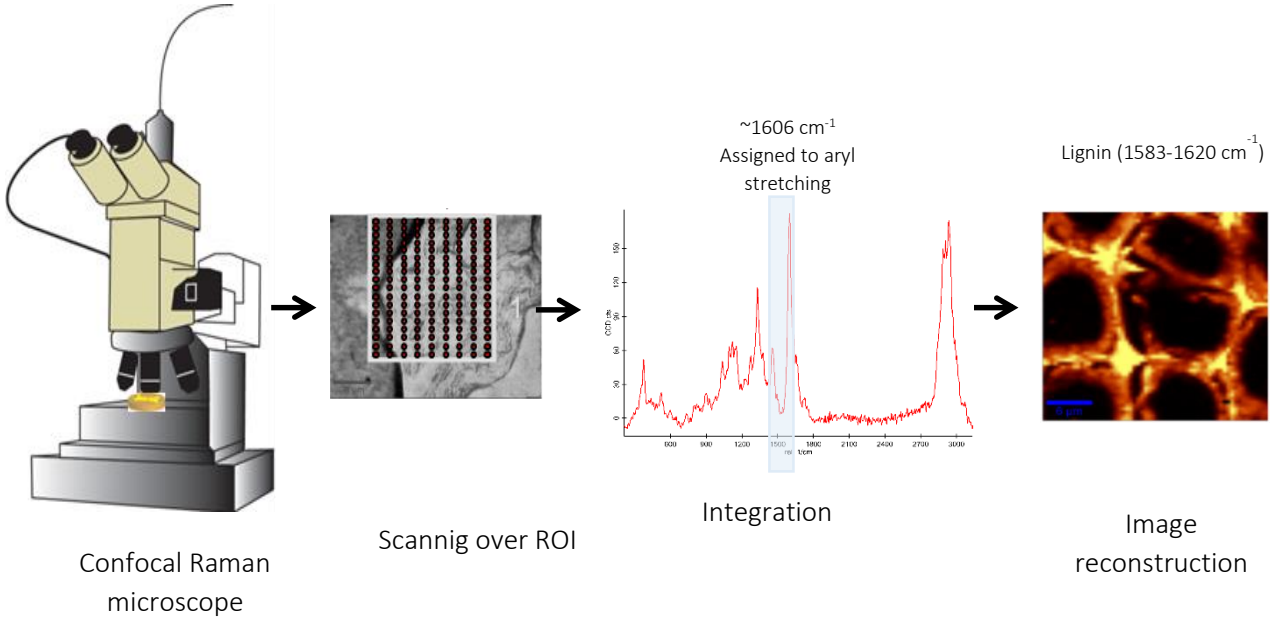

Figure 9. The basic principle of the Raman spectroscopy.

Furthermore, fluorescence has a competitive effect on Raman scattering, even though the fluorescence can be decreased by water (or oil) maceration or oxygen flushing as well as time gated Raman spectroscopy. However, the weak fluorescence is usually stronger than the Raman scattering (Lieber 2003). The maceration process was chosen by removing the lignin to decrease the fluorescence of the material (Saariaho, Jaaskelainen et al. 2004).

2.4.2 FT-IR spectroscopy

As the dispersive systems were superseded by the powerful FT-IR (Fourier-Transform-Infrared) spectrometers, IR spectroscopy progressed into a widely used analytical tool. One of the advantages of FT-IR spectroscopy is its capability to identify functional groups such as C=O, C-H or N-H. Most substances show a characteristic spectrum that can be directly recognized. FT- IR spectroscopy enables measuring all types of samples: solids, liquids and gases (Kataoka, Kondo 1998).

Infrared (IR) spectroscopy utilizes the infrared region of the electromagnetic spectrum. Infrared region is the light with a longer wavelength and lower frequency than visible light. The technique is mostly based on absorption. IR radiation induces vibrations in a functional group. This causes molecular vibration which absorbs radiation on a certain wavelength. The absorption is then detected on the whole spectrum of wave lengths. Absorbance bands originate from functional groups from the sample. Therefore, IR spectroscopy is mainly sensitive to organic functional groups (Kataoka, Kondo 1998).

2.4.3 Transmission Electron Microscopy (TEM)

TEM is short for transmission electron microscopy, which is specialized to use the high voltage electron beam to create images. The particle size is analysed by basic principle of electron beam scatter at particular atomic plane. Under high vacuum condition this electron beam can be free path move to optical detector. In addition many mathematical projection software are available to determine the particle size view along three dimension ways. We can have higher magnification than SEM so that the images of nano structures can be seen in a high magnification (Maurer 1990; Reza, Rojas et al. 2014).

2.4.4 Scanning Electron Microscopy (SEM)

SEM is a surface scan technique, from which you could get information on the sample surface, morphology, but by varying the detectors one could get general information on the surface composition. The scanning electron microscope contains an electron generating component called the gun, a column through which the electron beam travels, a series of lenses that shape the electron beam, then a series of pumps to keep the system under vacuum, which is also mostly used for the surface characterization and the magnification mechanism (Rout, Tripathy et al. 2001).

2.4.5 UV-Raman Microscopy

UV resonance Raman (UVRR) spectroscopy is one powerful method for detecting traces of UV absorbing components from the matrix, the Raman scattering maybe enhanced by several orders of magnitude when the exciting frequency is close to the electronic transitions of the molecule under investigation (Nuopponen 2004).

Ultraviolet Raman spectroscopy, a highly sensitive technique that enables in situ assessment of trace chromophores in polysaccharides and analysis of residual lignin, was employed in this study (Nuopponen 2004). The fluorescence that we encountered during the Raman spectroscopy does not present in the UV-Raman microscopy (Pandey, Vuorinen 2008).

3 Material and methods

As outlined from the section 1.2, one of the aims of this study is to characterize the fibre properties by using optical microscopy, TEM (transmission electron microscopy), Raman microscopy, infrared spectroscopy, SEM (scanning electron microscopy). For the chemical analysis of sugars, HPAEC (high-performance anion-exchange chromatography) was used.

All chemicals used in this thesis were research grade and purchased from Sigma-Aldrich, Finland. Instruments used belong to the Department of Forest Products Technology and the Nano-microscopy Centre in Aalto University as well as VTT Oy.

3.1 Materials

3.1.1 Source of raw materials

The wood material corresponds to four clones of willow hybrids: S1-Salix Myrsinofolia (Finland), S2 Karin (Sweden), S3 Klara (Sweden), S4 Salix schwerinii (Russia), which were obtained from VTT's Kyyjärvi plantation. These four years old willow trees were cut manually during the middle of October (17 th Oct, 2014), and classified as plant material ranging from the first to four years by checking the tree joint section. Experiments performed during this study were carried out in stem corresponding to the 3rd year of growth. Visual description of the willow plantation is shown in Figure 10.

Figure 10. VTT's willow plantation.

3.1.2 Raw material preparation

The willow plant material was processed immediately after the delivery. All the branches were brushed to remove lichen/grit and then being left to the freezer (-20 ^{o} C) for keeping fresh. Willow stems used in this thesis were manually debarked by scalpel after leaving the material into water (20^{o} C) for immersion overnight (Bedard, Laganiere 2009). Subsequently, the inner bark material was placed in the air-conditioning room (RH 35%, 20^{o}C) to dry overnight for further research. Finally, willow inner bark was grinded to a fine powder (1mm mesh) by a conventional Wiley mill grinding machine (USA /motor: Strömberg Oy).

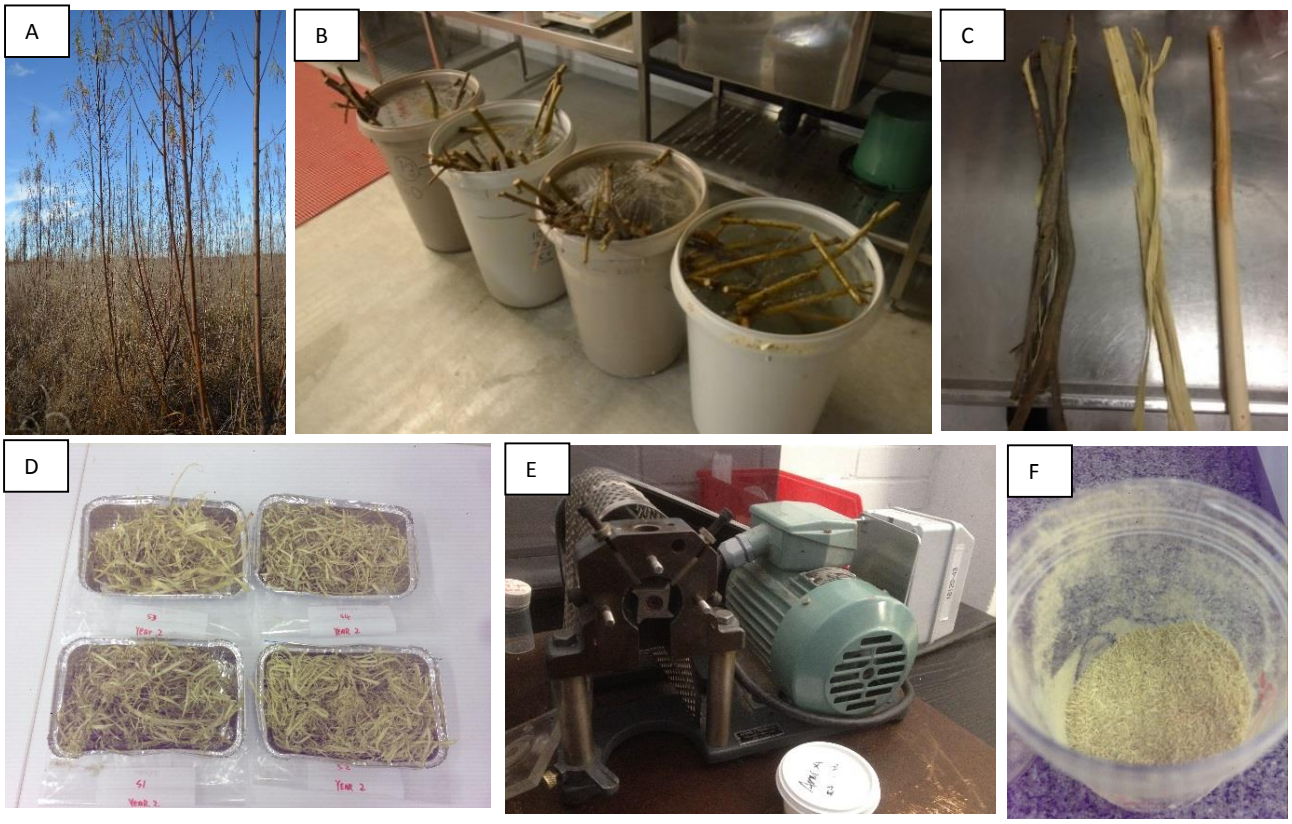

Figure 11. Preparation of the samples: (A) Original fresh sample, (B) Water infiltration pre-treatment, (C,D) Manual debarking, (E) Wiley grinding, (F) Willow inner bark powder.

3.2 Methods

Table2. Standard methods used in this study.

Analysis	Standard
Dry matter content of chips	SCAN-C 39:97
Lignin and carbohydrate content of wood and pulps	NREL/ TP 510-42618
Ash content of wood and pulps	NREL/ TP 510-42622
Acetone extractives content of wood and pulps	SCAN-CM 49:03
Disintegration for the inner-bark fibre after chlorite treatment	ISO 5263:1995 (E)

3.2.1 Chlorite treatment for fibre separation

The characterization of the basic fibre properties in the willow's inner bark and wood were done from material that underwent chlorite treatment to remove the lignin. Preliminary experiments performed showed that conventional cooking method (kraft cooking) is also a feasible procedure to obtain fibres.

Treatment with sodium chlorite ($NaClO_2$) under acetic acid condition was successfully applied by several research groups for removing lignin from plant materials (Kang, Jeun et al. 2007; Keshk, Suwinarti et al. 2006; Ahlgren 1971). Figure 12 briefly shows the chlorite treatment of the samples: 1.5g sodium chlorite and 0.5 ml acetic acid (99. 8%) were used to solubilize lignin in distilled water (70ml) at 80 °C for 5h in the water bath. Finally, the Metso Fibre-Lab machine was used to analyze the fibre properties after the fibre disintegration process (volume: 2L, number of revolutions: 30000r). The specific recipe for this treatment can be seen from Appendix 1.3.

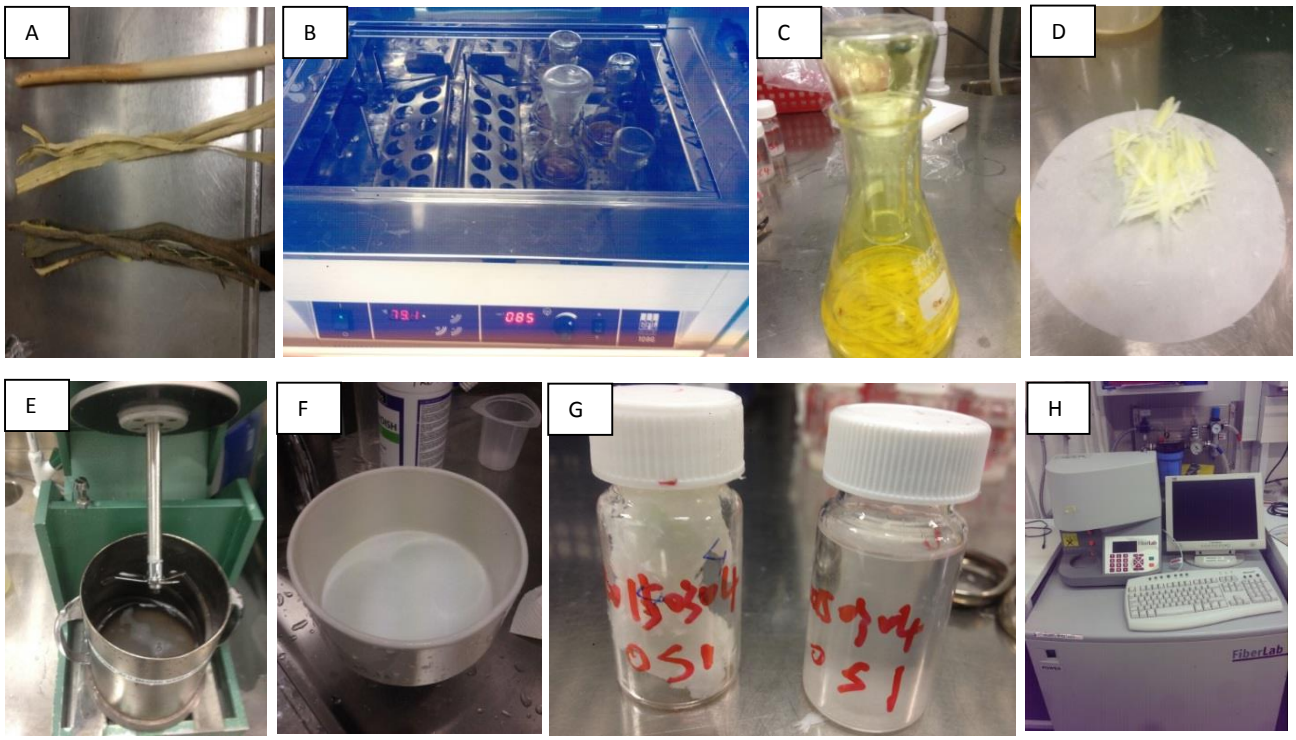

Figure 12. Process for the chlorite treatment: (A) Debarked willow inner bark and wood (bark to wood weight ratio in the tree stem was approximately 1:10 on dry basis), (B) Water bath (80° C), (C,D) Fibres after chlorite treatment, (E) Disintegration machine, (F) Vacuum filter, (G) Fibres, (H) Metso FibreLab analyzer.

3.2.2 Optical microscopy

Alcian blue and safranin dye were used to characterize the cell structure of inner bark cell wall. The samples were embedded in PEG (Polyethylene glycol, MW 2,050 $gmol^{-1}$) to obtain micro-sections of high quality (Gierlinger, Keplinger et al. 2012).

Figure 13. Process for the PEG embedding.

As shown in Figure 13, histological sections (8 μm thick) were obtained using microtome. The samples were dehydrated with ascending series of ethanol (70, 90, 94 and 100%), and then dyed with Alcian blue and

safranin, as Figure 14 shows. The Alcian blue dyes cellulose with blue color, while safranin dyes lignin with red.

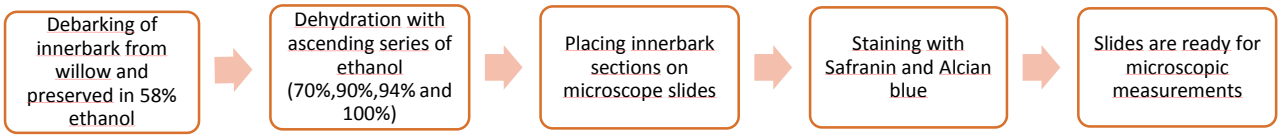

Figure 14. Process of dye staining with Alcian blue and safranin.

3.2.3 TEM

Debarked willow inner bark sections were stained with $KMnO_4$ (1% (w/v)) for 45 min, and dehydrated in ascending series of ethanol (58%, 70%, 80%, 90%, 99%). This staining method is specific for lignin and can improve the contrast between regions of variable lignin content within wood cell wall. The sample was embedded with resin (SpeciFix Resin), which is a transparent epoxy mounting system particularly suited for the mounting of small delicate specimens. Then Leica 125 Ultracut Microtome and Leica EM UC7 Ultramicrotome with cryo FC7 were used for trimming and polishing before using the TEM (FEI Tecnai 12) at an accelerating voltage of 120 kV (Bland 1971; Reza, Rojas et al. 2014).

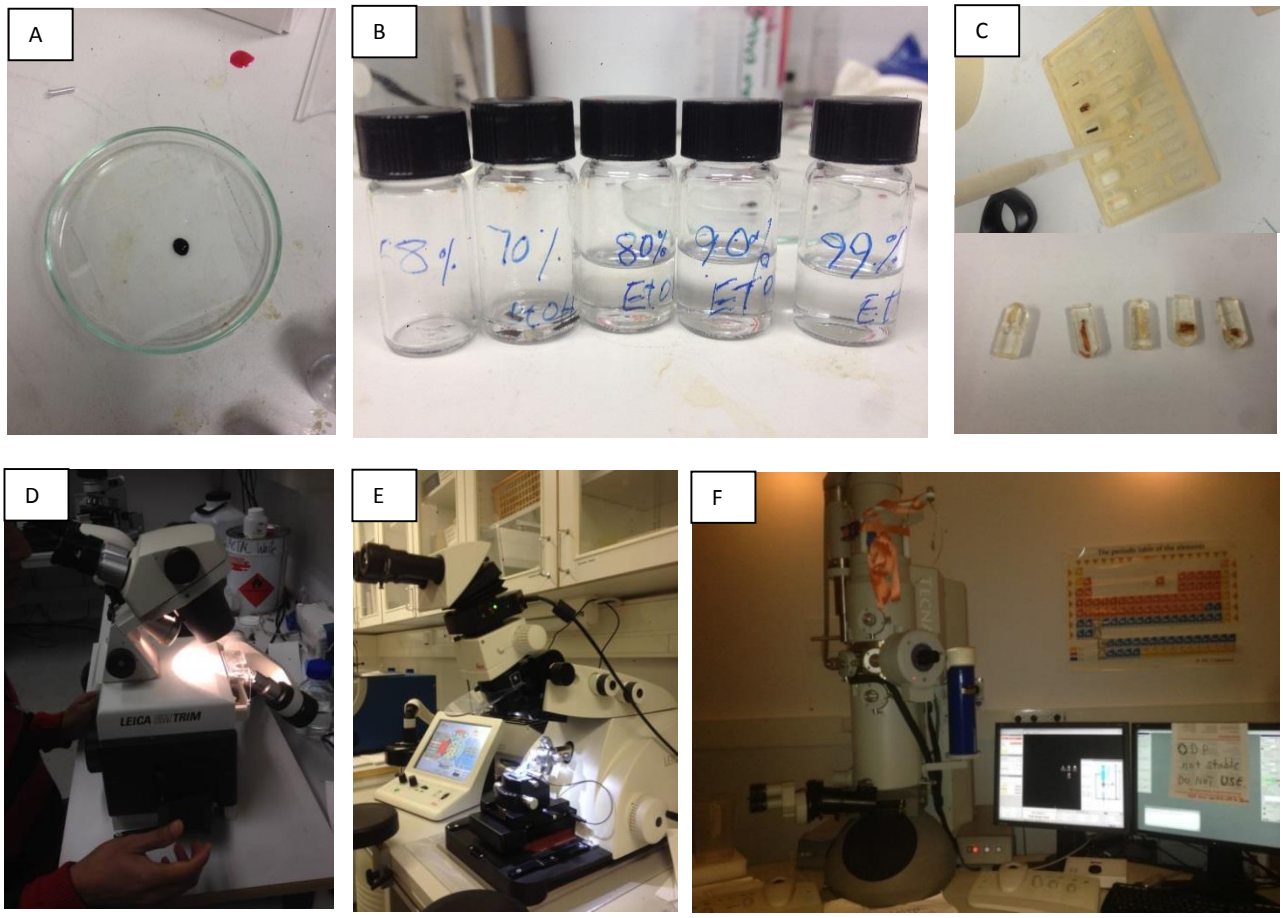

Figure 15. Process for using TEM: (A) $KMnO_4$ staining, (B) Ethanol dehydration, (C) SpeciFix Resin embedding, (D) Leica 125 Ultracut Microtome, (E) Leica EM UC7 Ultra microtome, (F) Cryo FC7/ CTEM (FEITecnai 12).

3.2.4 SEM

Scanning electron microscope (SEM) (Zeiss RIGMA VP) was used for analyzing the inner bark sclerenchyma fibre bundles from the cross section and the longitudinal section with the accelerating voltage of 3 kV. The surface was dry-cut by using a microtome (Leica EM UC7) at room temperature. An Emitdec K100X sputter coater was used to apply gold to the samples before SEM analysis (Antikainen, Paajanen et al. 2014).

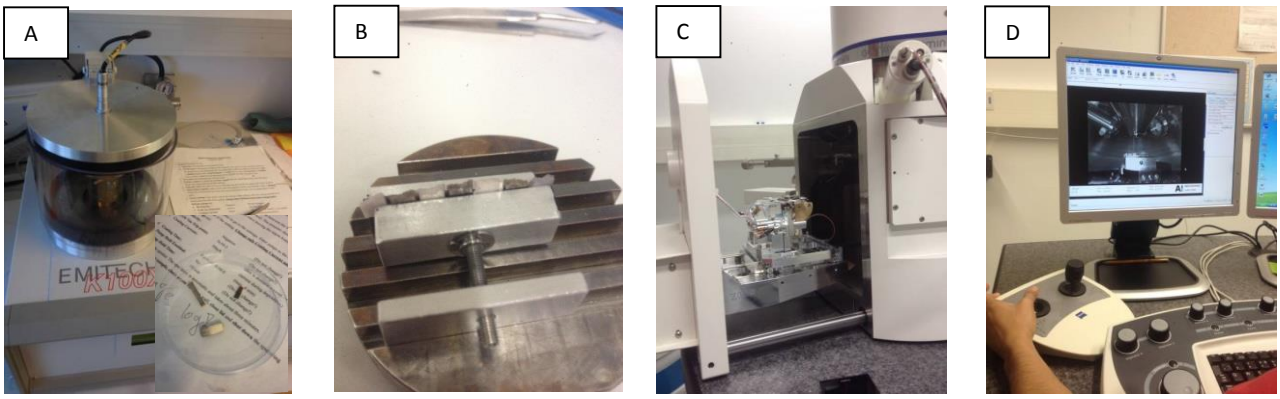

Figure 16. Process for using SEM: (A) Emitdec K100X sputter coater machine, (B) Sample fixing, (C, D) Scanning electron microscope (SEM) (Zeiss RIGMA VP).

3.2.5 Chemical analyses

The chemical analysis of the samples comprised four major experimental parts: extractives determination, sugar analysis by using HPAEC, lignin measurement and ash determination. The Klason and acid-soluble lignin and carbohydrate contents were determined on the extracted materials. All the procedures strictly followed earlier publised standard methods (Sluiter et al, 2011). In total, the four studied willow inner bark and wood samples were analyzed seven and three times for getting reliable result respectively.

3.2.5.1 Extractives

The inner bark powders were extracted with acetone (polar solvent) in a Soxhlet setup by following the standard SCAN-CM 49:03 (2003). The samples (approx. 10g of each) were extracted with 300ml of acetone for 6 hours. After extraction the solvent was evaporated by using the rota-vapour. After the volume of the mixture was below 50ml the sample was transferred into pointed shaped flask (50ml) for further evaporation before the residue was shifted to an aluminum container. The extracted sample was taken from the extraction thimble for the sugar determination. The hydrophilic extractives were dried at lower temperature to prevent oxidation (40^{o} C for 2 hours) before further analysis with respect to its composition.

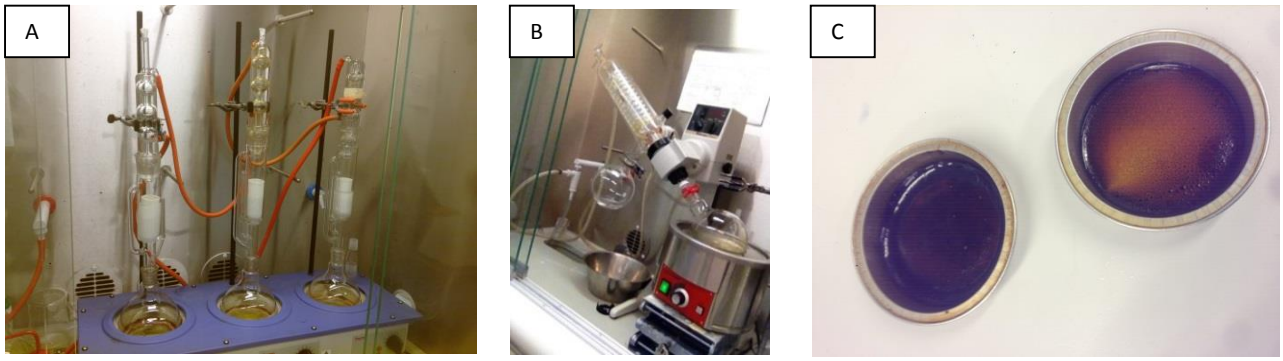

Figure 17. Process for extractives determination: (A) Soxhlet extraction, (B) Rota-vapour, (C) Hydrophilic extractives.

3.2.5.2 Carhohydrates

The sugar analysis started by measuring the dry matter content of the extracted powder. Dried powder of extracted inner bark (300 ± 15mg) was placed into a test tube, and slowly mixed with 3 ml 72% H_2SO_4 (using pipette tips with filters). Subsequently, the sample was incubated at 30 ± 3 °C in a water bath for 60 minutes (mixing every 5 minutes). Distilled water was added (84ml) before being transferred into a Duran bottle. Finally the sample was hydrolysed at 121°C in autoclave for two hours (Sluiter et al., 2011). After cooling at room temperature, the hydrolysed sample was diluted and filtered through a 0, 2µm filter. Thereafter the sample was analysed by high-pressure anion exchange chromatography (HPAEC, Dionex ICS-3000 Ion Chromatography System) against calibration standards (Standards 50mg/L, 2 injections/sample). The column was CarboPac PA 20 with the flow 0.4ml/min of water as eluent. The four willow clones were analyzed seven times for inner bark and three times for wood samples to obtain more representative result, check Appendix 4.2 for the specific recipe of those ten sets of data.

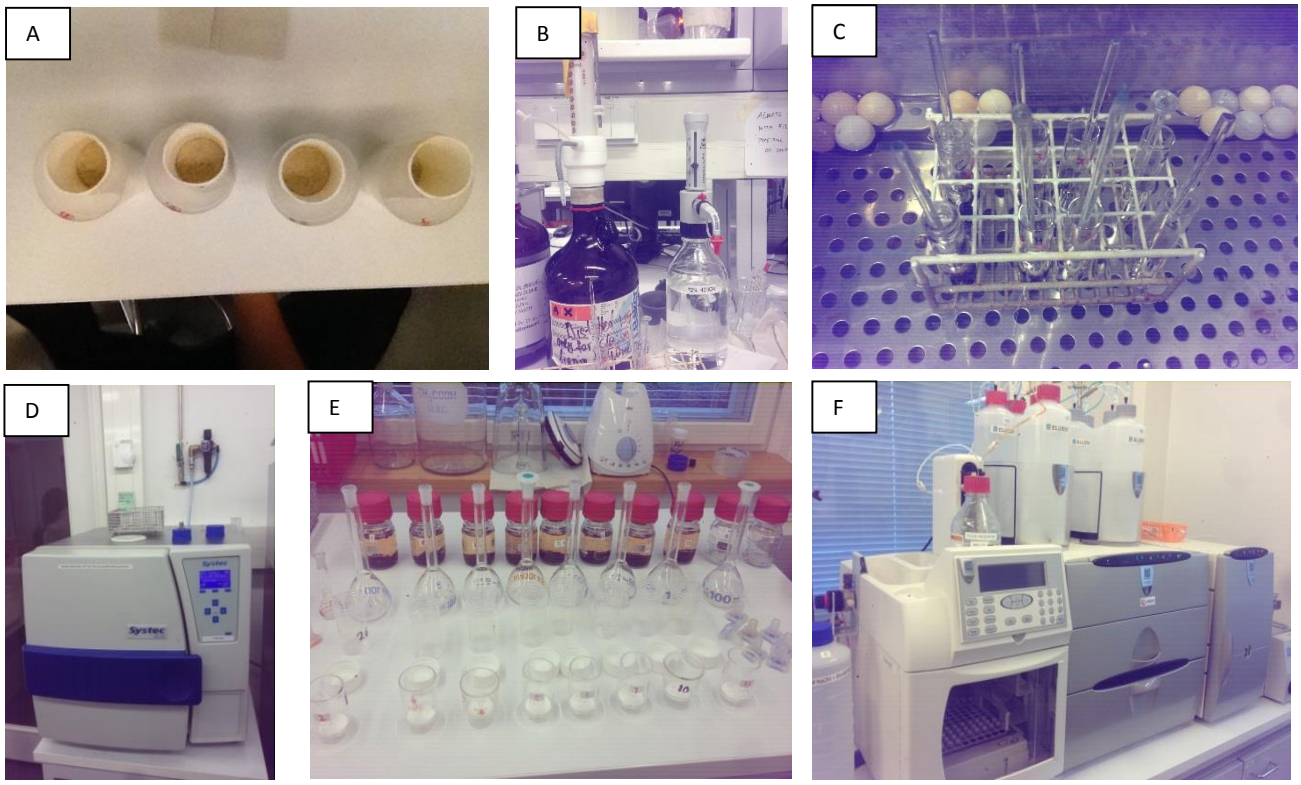

Figure 18. Process for sugar analysis: (A) Extracted powder, (B,C) Sulphuric acid and water bath, (D) Autoclave, (E) Sample dilution and cylinder filtering, (F) HPAEC (high-pressure anion exchange chromatography).

The sugar composition of hydrolysates solution was determined by HPAEC analysis. The samples were prepared by passing the decanted liquid through a 0.2 µm filter into an autosampler vial. The concentration of sugars was calculated based on the concentration of the corresponding monomeric sugars and the following equation (anhydrosugar correction factor for xylose and arabinose was 0.88 and for glucose, galactose, and mannose it was 0.9).

$$C\ \text{anhydro} = C\ corr \times Anhydro\ correction$$

$$\%\ Sugar\ ext\ free = \frac{C\ anhydro \times V\ filtrate \times \frac{1g}{1000\ mg}}{OD\ Wsample} \times 100$$

$$\%\ Sugar\ as\ received = (\%\ Sugar\ ext\ free) \times \frac{(100 - \%\ Extractives)}{100}$$

Where:

$C_{anhydro}$ = concentration of the polymeric sugars

C_{corr} = concentration of the corresponding monomeric sugars

Anhydro correction = Anhydro correction of 0.88 (or 132/150) for C-5 sugars (xylose and arabinose) and a correction of 0.90 (or 162/180) for C-6 sugars (glucose, galactose, and mannose)

$V_{filtrate}$= volume of filtrate, 86.73 ml

ODW_{sample} = oven dry sample for sugar analysis

% Extractives = percent extractives in the prepared biomass sample

3.2.5.3 Lignin

Acid-soluble lignin

Dissolved lignin was determined on the combined filtrate by measuring the absorbance at 206 nm using a UV-vis spectrophotometer (Shimadzu). The background was deionized water or 4% sulphuric acid. The hydrolysis liquor aliquot obtained from the sugar analysis step was diluted with water to bring the absorbance into the range of 0.7-1.0.

$$\%\ ASL = = \frac{\text{UVabs} \times \text{Volume filtrate} \times \text{Dilution}}{\varepsilon \times \text{ODW sample} \times \text{Pathlength}} \text{ X } 100$$

$$\%\ Lignin\ ext\ free = \%\ AIL + \%\ ASL$$

$$\%\ Lignin\ as\ received = (\%\ Lignin\ ext\ free) \times \frac{(100 - \%\ Extractives)}{100}$$

Where:

UV abs= average UV-Vis absorbance for the sample at the appropriate wavelength

Volume hydrolysis liquor= Volume of filtrate, 86.73ml

Dilution= (Volume e_{sample} + Volume $_{diluting\ solvent}$) / Volume $_{sample}$

ε= Absorptivity of biomass at specific wavelength

ODW_{sample} = weight of sample in milligrams

Pathlength = pathlength of UV-Vis cell in cm

% Extractives = percent extractives in the prepared biomass sample

Insoluble Klason lignin

As Figure 19 shows, the insoluble Klason lignin was determined as the mass of the hydrolysis residue after drying at 105°C overnight until a constant weight was achieved. On the following day, the samples were removed to cool down in a desiccator. Finally the crucibles were cleaned with 30% H_2O_2 and 98% H_2SO_4 for further use. The Klason lignin content was reported as percentage on the original sample.

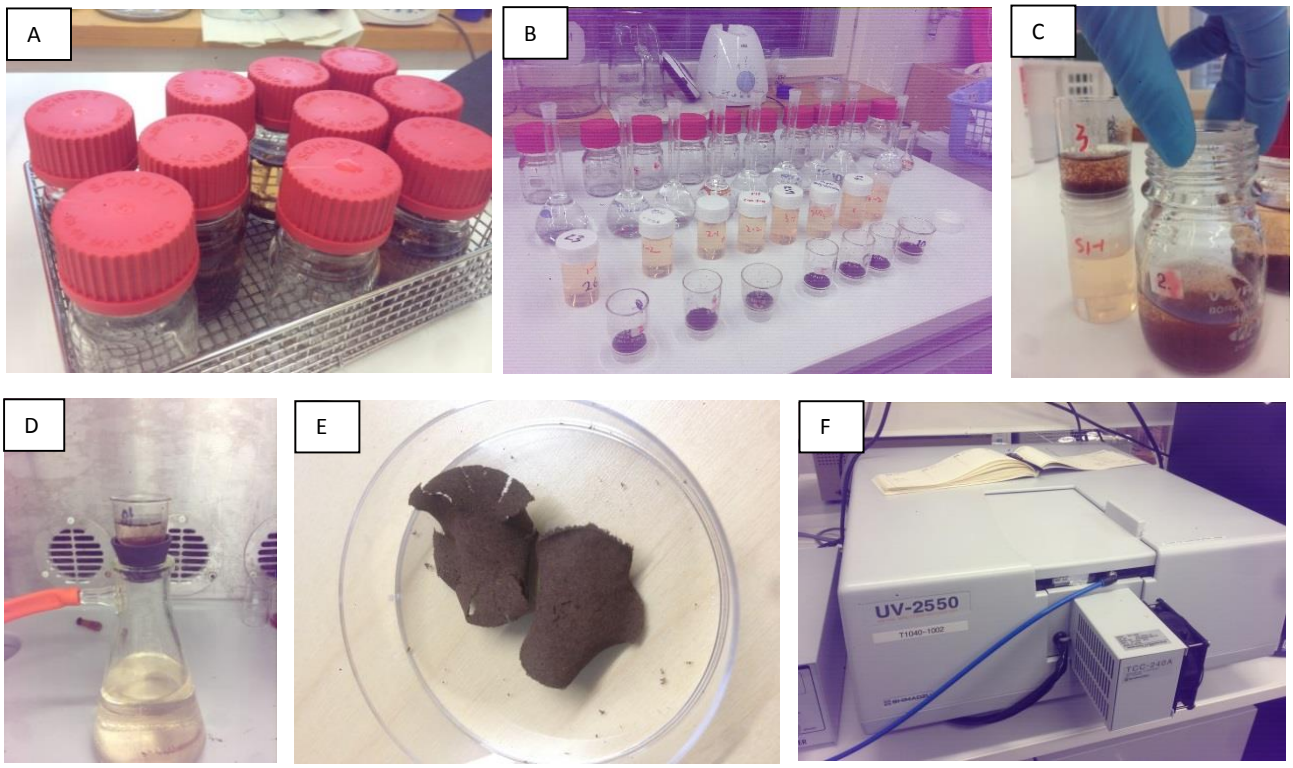

Figure 19. Process of lignin determination: (A-D) Lignin filtering, (E) Lignin, (F) UV-spectrophotometer.

3.2.5.4 Ash

The debarked willow inner bark and wood powder were used during the ash determination, and slowly carbonized by using a muffle-oven at 575 ° C for 180 minutes until the sample turned into white ash to constant weight. Finally, the crucibles with ash were left to the oven and desiccator for cooling down before weighing the crucibles and ash to nearest 0.1 mg (GUSTAFSSON, NJENGA 1988). Check the specific data from Appendix 4.1.

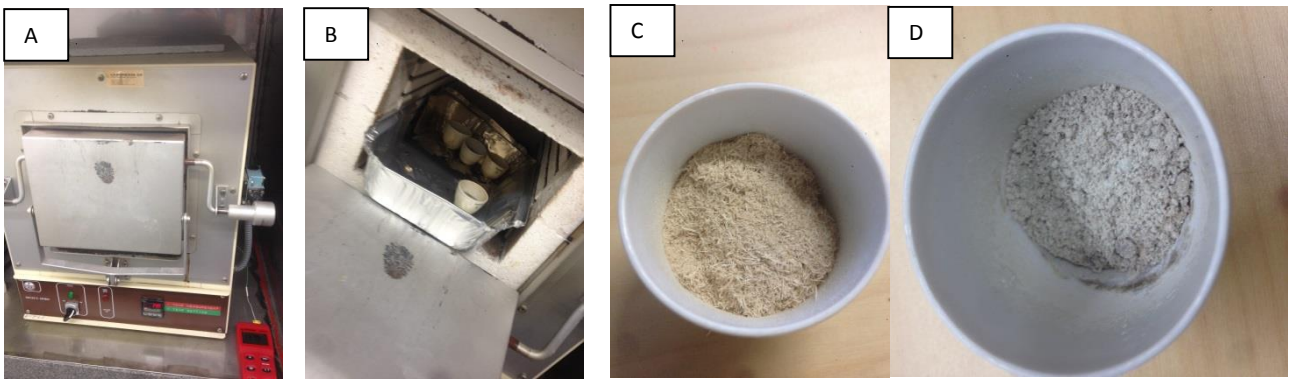

Figure 20. Process for ash determination: (A, B) Furnace, (C, D) Powders before and after the furnace.

3.2.6 FTIR spectroscopy

Infrared (IR) spectroscopy was used for the chemical characterization of the extractives and salicin. Inner bark mixed in potassium bromide (KBr) was prepared as pellets for IR-spectroscopy determination.

As shown in Figure 21, starting material and the glassware were dried in an oven and cooled down in the desiccators. Firstly 1-2 mg of the oven dry sample and 300 mg of KBr were added to the vibration mill capsule

for 30-60 seconds. Finally the mixture was pressed into a pellet with 3000 psi pressure for two minutes. The IR spectrum was recorded immediately after the preparation of the pellet (Michell 1989).

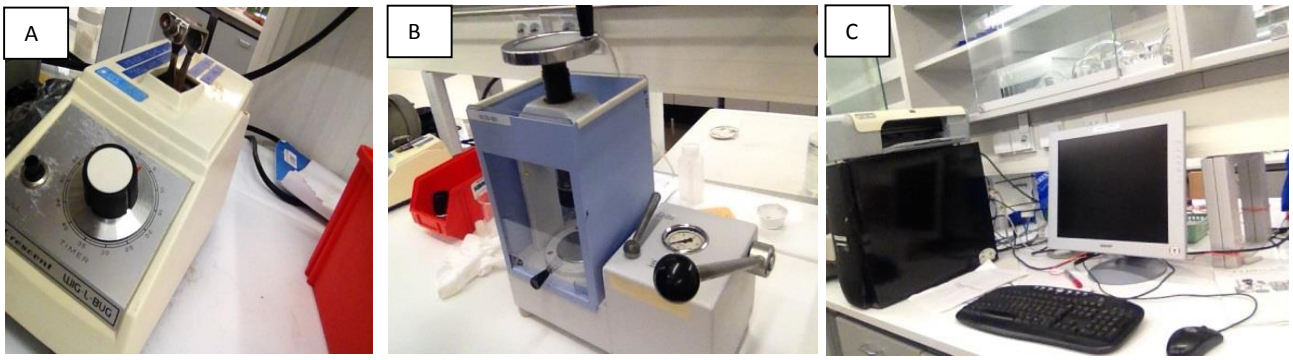

Figure 21. Process for IR spectroscopy: (A) Mixing machine, (B) KBr pellet compressing machine, (C) FTIR spectrometer.

3.2.7 Raman microscopy

The willow inner bark samples were measured on a macerated material by using a 1:1 glacial acetic acid with hydrogen peroxide at 40° C during 48 hours for cell dissociation pretreatment, which was used to decrease the fluorescence of the samples (Kuznetsov, Kuznetsova et al. 2009).

Raman spectra were recorded by using the alpha 300 R confocal Raman microscope (Witec GmbH, Germany) equipped with a piezoelectric scanner at ambient conditions for analyzing the lignin distribution and chemical bonding from the inner bark extractives as well as the inner bark fibres. A frequency doubled Nd: YAG laser (532 nm, 40 mW) was focused onto the sample using a 60× (Nikon, NA = 0.95) air objective. The excitation laser was polarized horizontally and the spectra were acquired by using a CCD (DU970N-BV) behind a grating (600 grooves/mm) spectrograph (Acton, Princeton Instruments, Inc., Trenton, NJ). An integration time of 0.3 s was used for collecting each spectrum. The baseline correction was performed with WiTec project 2.10 (Witec GmbH, Germany) by using a fifth order polynomial. Further smoothing of the spectra was performed by using OriginPro 9.0.0 (OriginLab Corporation, Northampton, MA). Raman images were constructed by integrating characteristic bands.

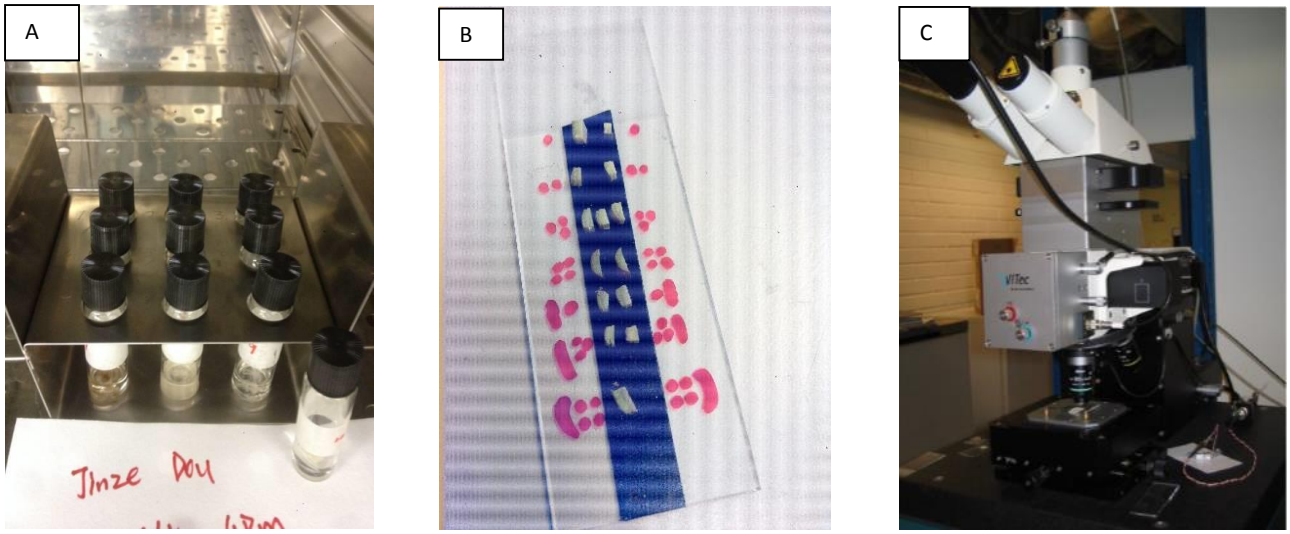

Figure 22. Process of sample preparation for Raman spectroscopy: (A) Maceration (48 h at 40° C for cell separation), (B) Samples ready for Raman spectroscopy, (C) Raman microscope.

3.2.8 UV-Raman microscopy

UVRR spectra were obtained with a Renishaw 1000 Raman spectrometer coupled with an Innova 90C FreD frequency doubled argon ion laser (Coherent) and a Leica DMLM microscope. Spectra were collected using a 40 X deep UV objective (OFR). An excitation wavelength of 244 nm was used in all measurements. The detector was an UV coated CCD camera and a diffraction grating of 3600 grooves per millimeter was used. Samples were rotated during the measurements in order to avoid changes from the sample due to the focused UV light in the sample and to obtain averaged spectra. The spectra were collected at 200–2400 cm^{-1} under resolution of 7 cm^{-1} for 60 secs.

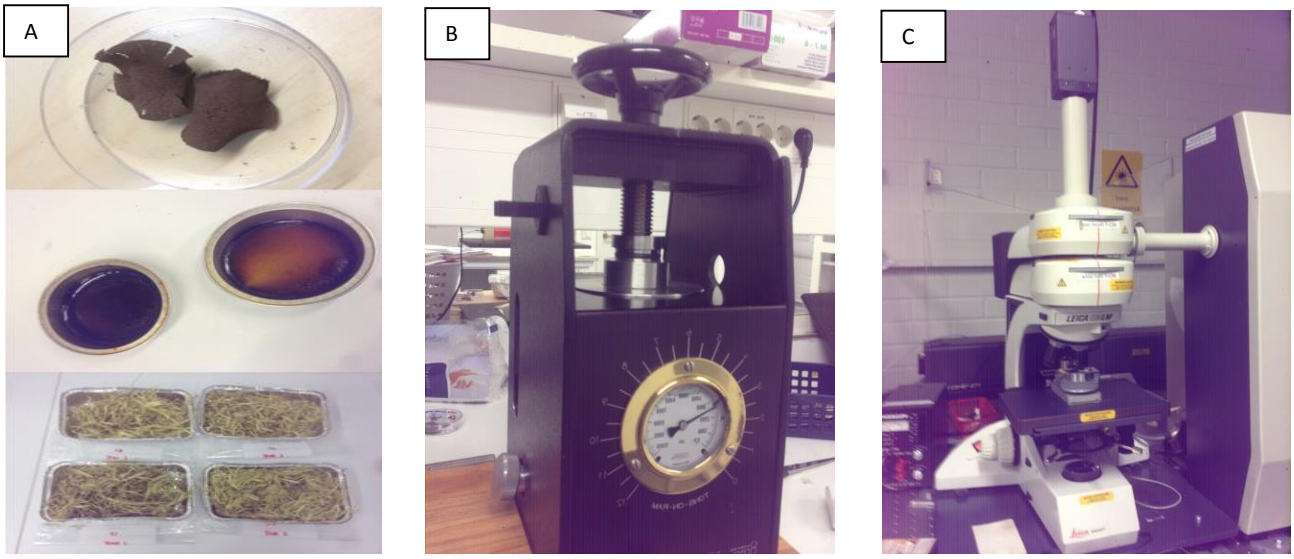

Figure 23. (A) Inner bark based samples (original/ extracted / extractives/ lignin), (B) Compressing machine, (C) UV-Raman spectrometer.

4 Results and Discussions

4.1 Inner bark fibres

4.1.1 Fibre properties

Prior to measuring the bark fibre properties, several industrial methods to remove lignin were tested for individual fibre separation, namely kraft cooking, alkaline hydrogen peroxide cooking and neutral sulphite anthraquinone cooking. Preliminary results shown in Appendix 7.4 suggest that kraft cooking is a most suitable cooking method to obtain intact high-quality fibres from willow inner bark. However, the procedure chosen to obtain fibres for its physical characterization was chlorite treatment that is commonly used for isolation of holocellulose (cellulose and hemicelluloses) from lignocellulosic plant materials.

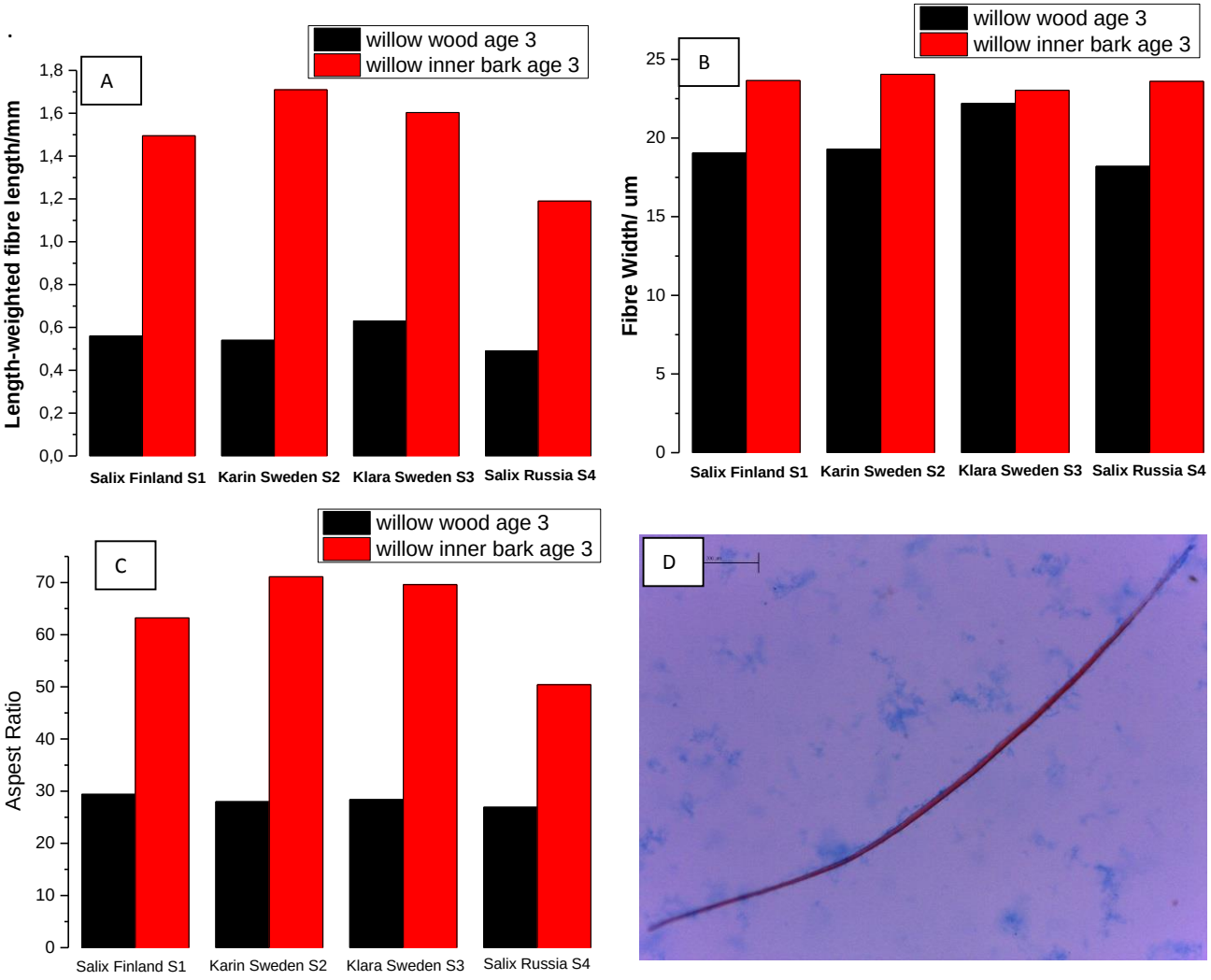

Figure 24. Fibre properties of willow inner bark and wood: (A) Length-weighted comparison, (B) Width comparison, (C) Aspect ratio comparison, (D) Optical microscope image of a single fibre from inner bark Hybrid willow 'Klara' – Sweden S3.

Figure 24 above and Table 3 below show the main fibre properties of the wood and inner bark of the four studied willow clones. In general, the inner bark fibres were around 2-3 times as long as the wood fibres. On the other hand the inner bark fibres were slightly wider than the wood fibres. Thus the aspect ratio of the inner bark fibres was double in comparison with the wood fibres. Among the clones studied Karin (S2) showed

the best physical properties (length and aspect ratio) while the inner bark fibres from Salix Russia clone (S4) showed the lowest curl and kink indices due to the shorter fibres (Figure 26). In comparison with the narrow length and width distributions of the wood fibres, the length and width distribution of the inner bark fibres were broader, as Figure 25 illustrates.

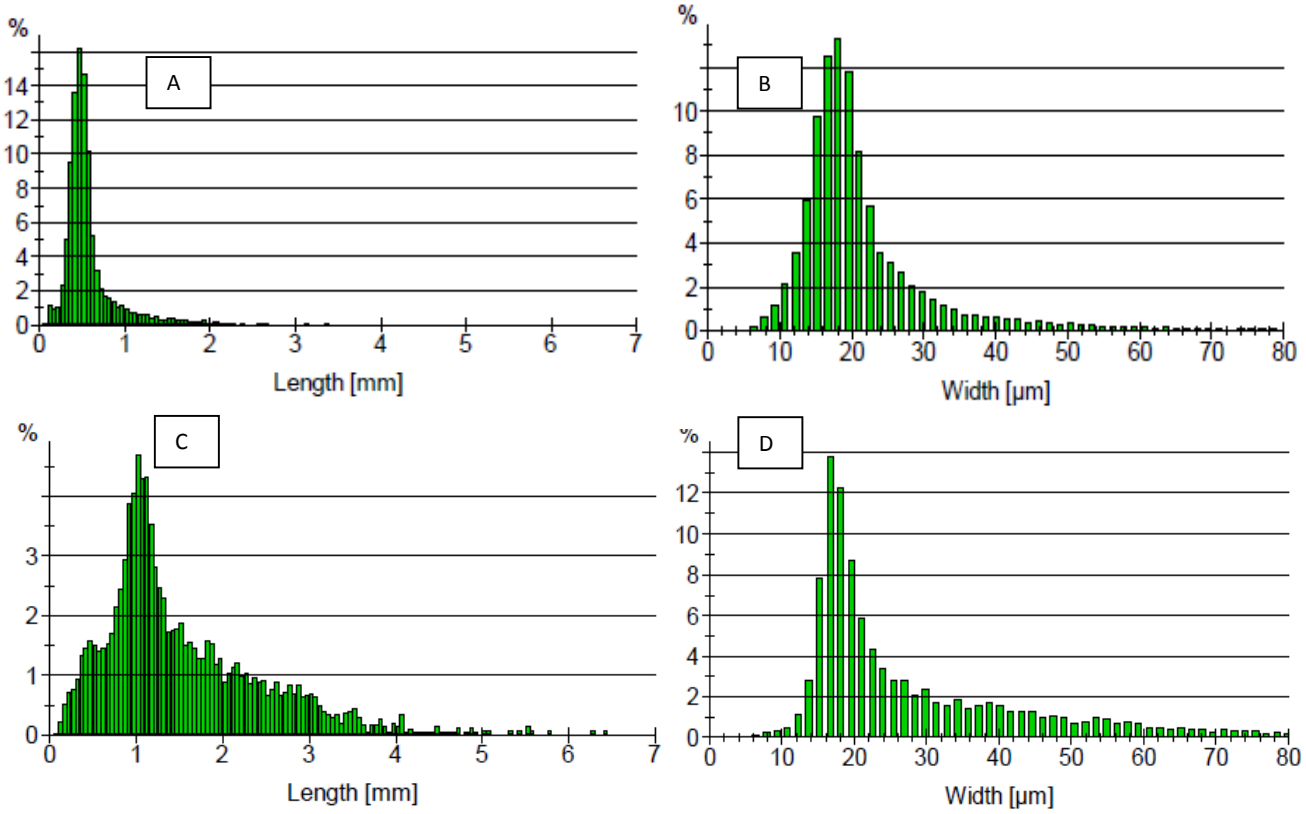

Figure 25. The histogram distribution of S2-Karin: (A) Wooden histogram length distribution, (B) Wooden histogram width distribution, (C) Inner bark histogram length distribution, (D) Inner bark histogram width distribution.

In general, the inner bark fibres had relatively low curl and kink indices. Because the willow wood fibres were so short, they even less curly and contained less kinks (Figure. 26, Table 4). Theoretically it is also possible that the inner bark fibres were more affected by the chlorite treatment than the wood fibres (Kang, Jeun et al. 2007; Keshk, Suwinarti et al. 2006; Ahlgren 1971).

Table 3. Mean values of main fibre properties (length, width, aspect ratio) of the willow clones (more details in Appendix 1.3).

Material	Fibre length (mm)		Fibre width (µm)		Aspect ratio	
	Wood	Inner bark	Wood	Inner bark	Wood	Inner bark
Salix Finland S1	0.56	1.50	19.1	23.7	29.4	63.2
Karin Sweden S2	0.54	1.71	19.3	24.1	28.0	71.1
Klara Sweden S3	0.63	1.60	22.2	23.0	28.4	69.6
Salix Russia S4	0.49	1.19	18.2	23.6	27.0	50.4

Table 4. Kink and curl values of the wood and inner bark fibres of the studied four willow clones.

Material	Curliness (%)		Kink index (m^{-1})	
	Wood	Inner bark	Wood	Inner bark
Salix Finland S1	2.30	7.55	46	277
Karin Sweden S2	2.35	9.55	58	328
Klara Sweden S3	2.30	8.93	51	330
Salix Russia S4	2.15	6.80	42	275

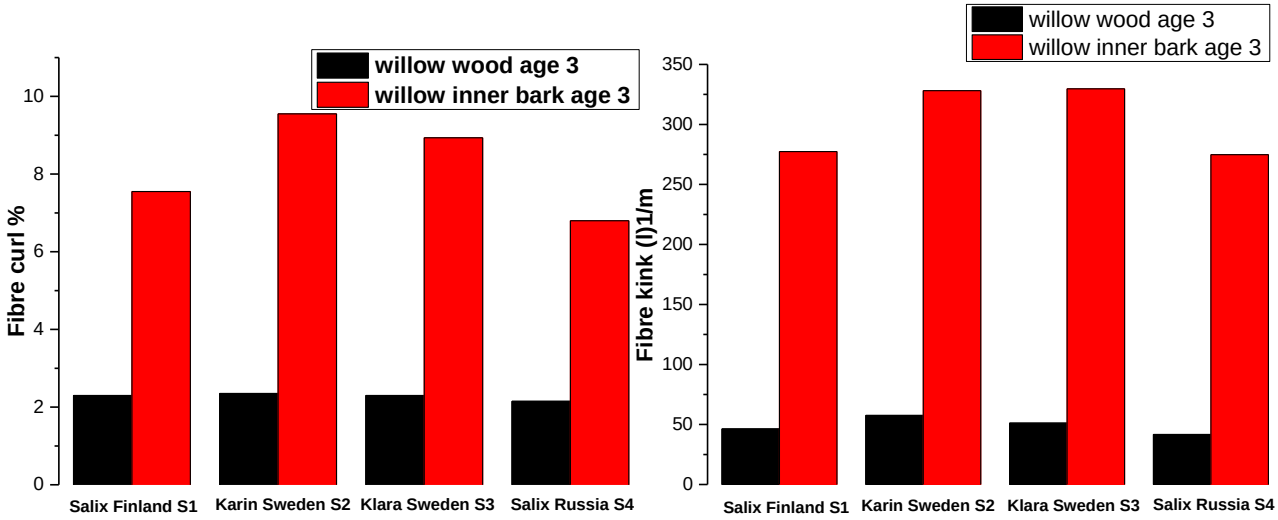

Figure 26. Curliness and kink distribution for the four studied willow.

For comparison, Table 5 shows basic fibre properties of typical Finnish softwood and hardwood species as well as some common annual plants. Here it should be pointed out that there are large variations in fibre dimensions within species and also within single plant (Groom, Shaler et al. 2002; KIBBLEWHITE, BAWDEN et al. 1991). Apparently, the willow wood fibres at this stage of growth are shorter (40%) compared to other hardwood species. On the contrary, the willow inner bark fibres have excellent characteristics (length, width, aspect ratio) in comparison with hardwood wood fibres (Figure 27).

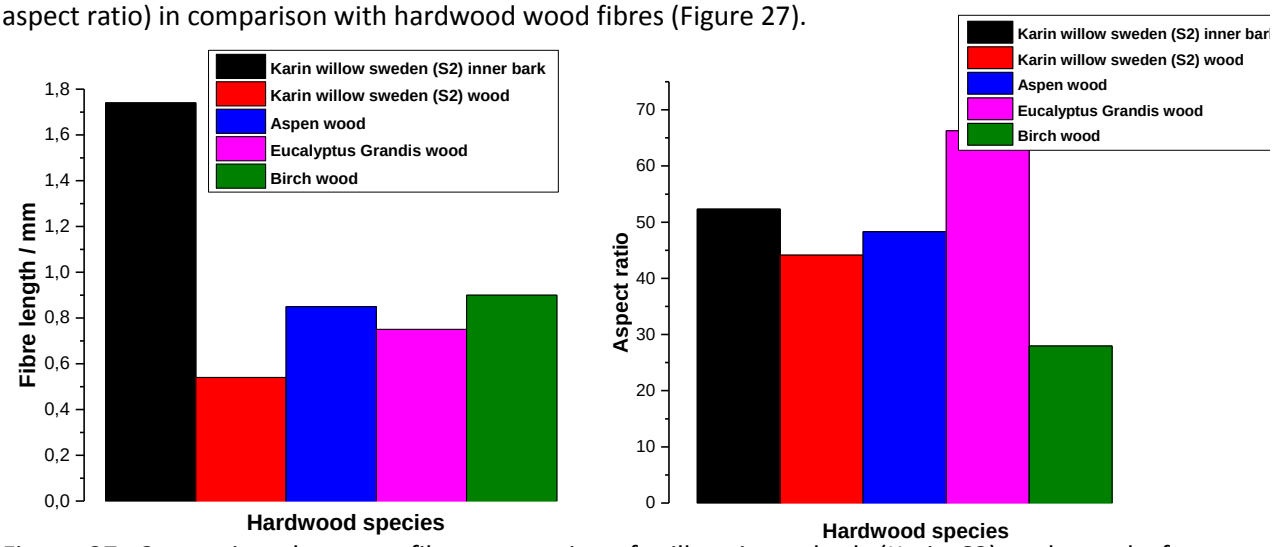

Figure 27. Comparison between fibre properties of willow inner bark (Karin S2) and wood of common hardwood species.

Table 5. Typical fibre basic properties of Finnish softwood and hardwood (Henricson, 2004).

Material	Species	Latin name	Fibre length (mm)	Fibre width (µm)	Aspect ratio
Softwood	Norway spruce	Picea abies	3.4	31	110
	Scots pine	Pinus silvestris	3.1	35	89
	European larch	Larix deciduas	3.5	38	92
	German spruce	Abies alba	3.7	38	97
	Ponderosa pine	Pinus ponderosa	3.6	42	86
	Redwood	Sequola sempervirens	6.1	53	115
	Western hemlock	Tsuga heterophylla	3.9	40	98
Hardwood	Silver birch	Betula pendula	0.9	17.2	52
	Eucalyptus	Eucalyptus grandis	0.75	17	44
	American aspen	Populus tremuloides	0.85	17.6	48
	Karin willow Sweden inner bark (S2)	Salix	1.71	25.8	66
	Karin willow Sweden wood (S2)	Salix	0.54	19.3	28

4.1.2 Sclerenchyma fibre bundles

Figure 28 shows the sclerenchyma bundle distribution in tangential (A) and transverse sections (B) at the border of inner bark and wood. Due to their high lignin content, the bundles are stained red by safranin while the surrounding cells with low lignin content are stained blue by the Alcian blue dye. In the tangential section, the phloem rays are also observed in both wood and inner bark.

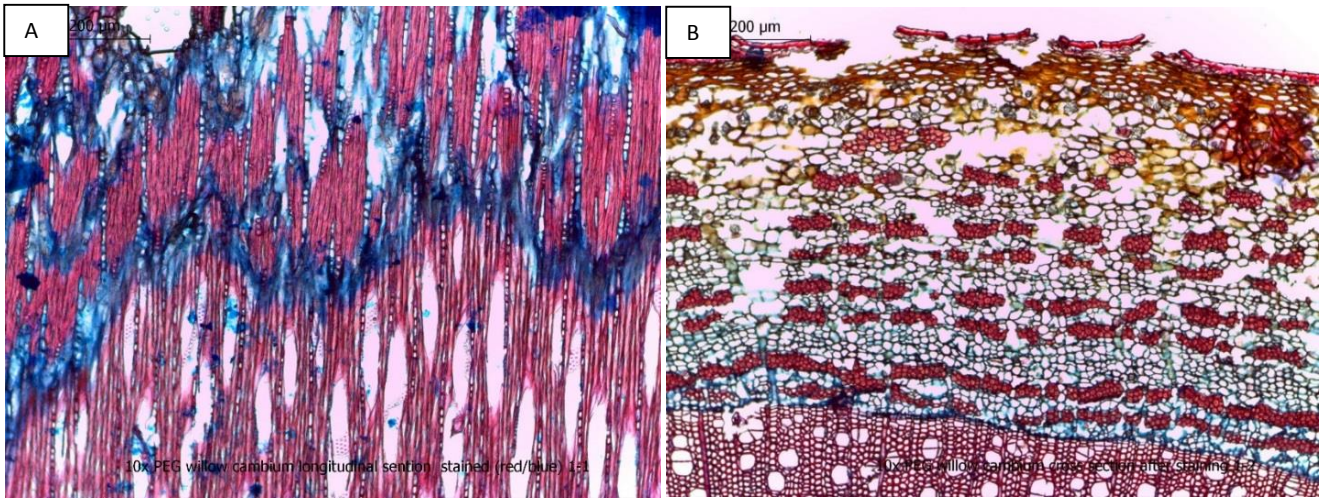

Figure 28. (A) Tangential section of willow wood and inner bark showing the sclerenchyma fibres (red color) of inner bark and also phloem rays (vertically arranged cells with small lumina), (B) Cross section of willow bark and wood showing the sclerenchyma bundles (red color) in the inner bark.

Annual growth layers, similar to annual rings in wood, were found in the inner bark of willow (Figure 29). Thus three concentric layers of fibre bundles were present in the inner bark of a three-year old willow tree. These growth rings of the sclerenchyma bundles were observed both by SEM (Figure 29B) and optical microscopy (Figure 29A).

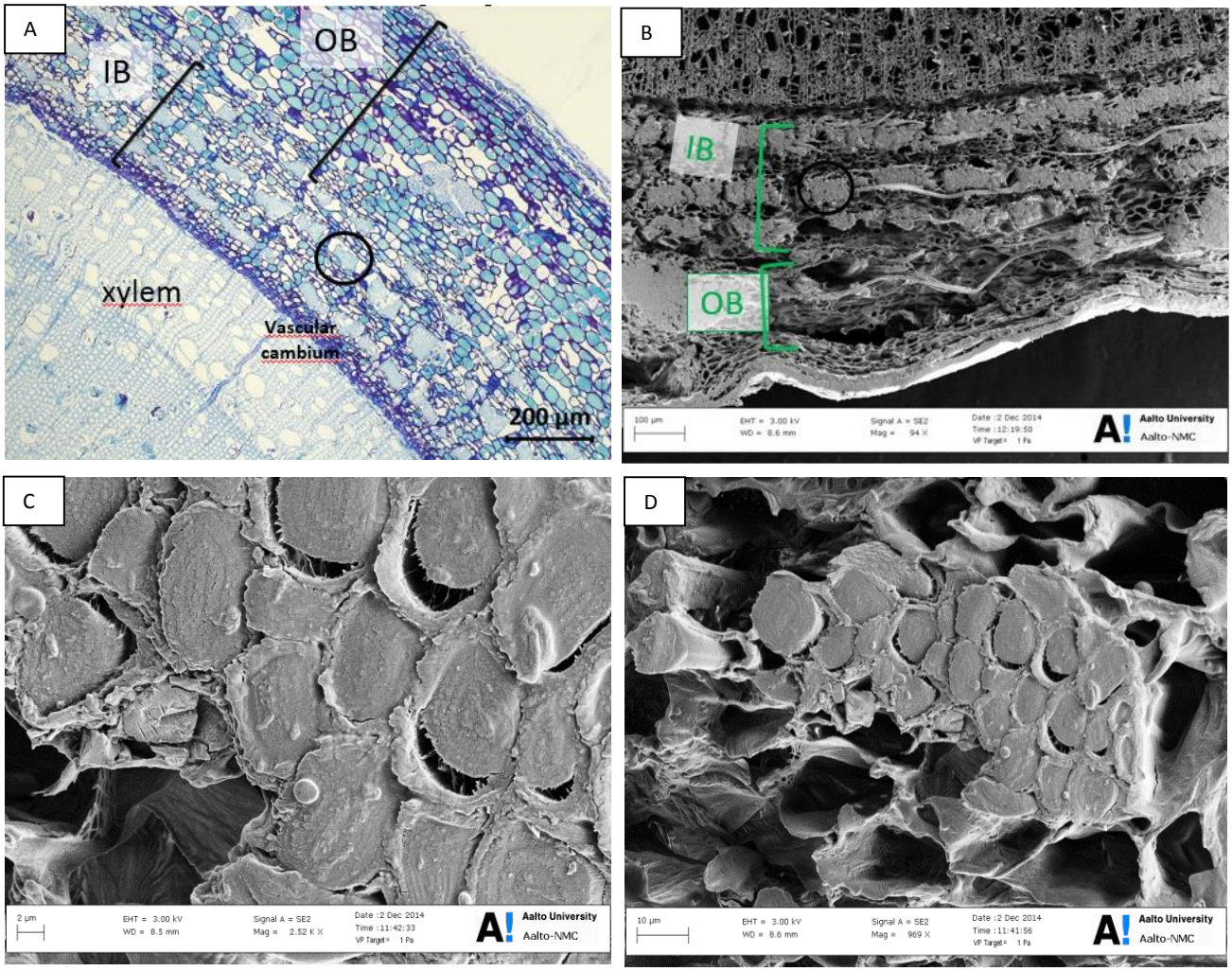

Figure 29. (A) Optical microscope image of cross section of willow (Salix Finland S1) wood and bark stained by toluidine blue showing the xylem, outer bark (OB), inner bark (IB) and fibre bundles (circle) distributed in the inner bark, (B-D), SEM of the same sample showing the fibre bundles in various magnifications.

No big differences were observed in the structure of the inner bark between the willow clones. Supporting optical microscopy and SEM images can be found in Appendices 2 and 3.

4.1.3 Fibre wall

TEM of wood sections that stained with $KMnO_4$ is frequently used for studying the lignin distribution in the cell wall. According to the degree of lignification, the different cell wall layers show distinct contrast and can easily be visualized with TEM (Bland 1971; Reza, Rojas et al. 2014).

Figure 30 shows the ultrathin cross section of willow inner bark sclerenchyma fibres after $KMnO_4$ staining. The middle lamella showed high contrast after $KMnO_4$ staining due to high concentration of lignin in this area.

Similarly, the lignin content varied between the different layers from the middle lamella to the lumen area. Depending on the clone and sample 3-8 layers could be identified in the inner bark cell wall (Figure 30A, Appendix 2). In comparison, TEM images of normal wood cell wall reveal typically only three different layers (S1, S2 and S3, Figure 30B).

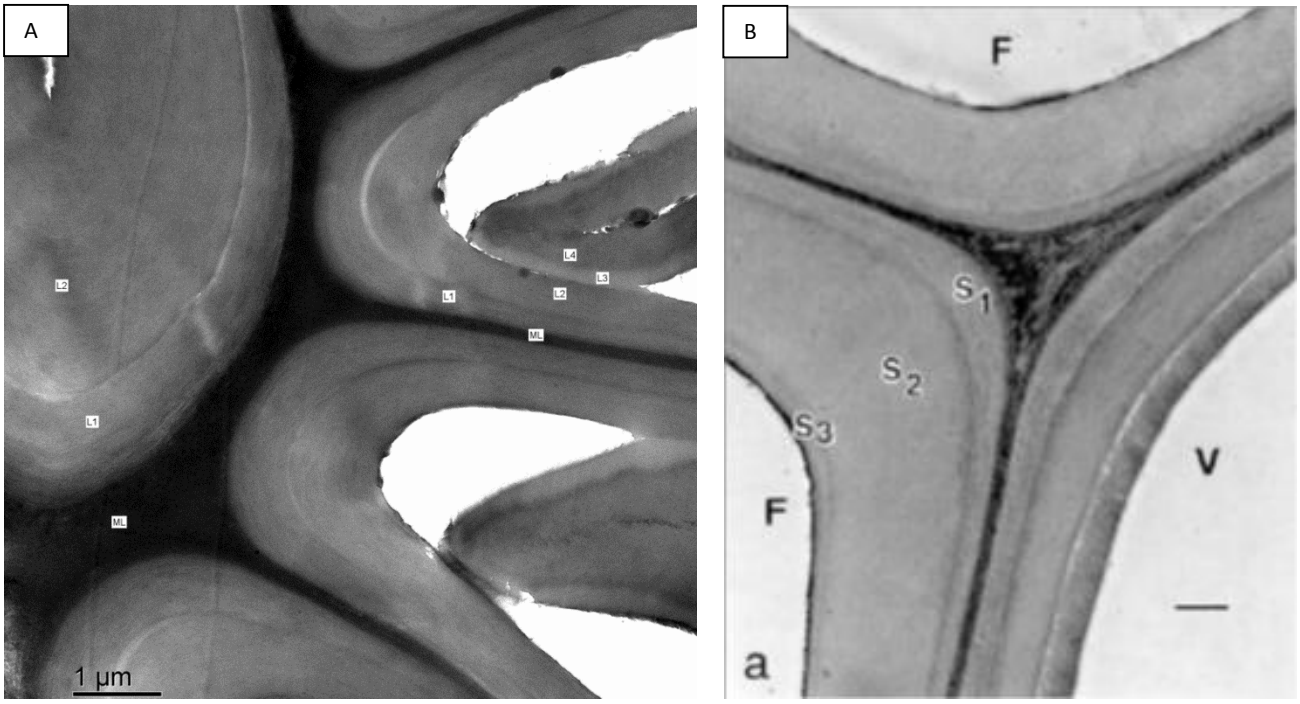

Figure 30. (A) TEM image of ultrathin cross section of S4 Salix Russia inner bark sclerenchyma fibres after $KMnO_4$ staining, (B) TEM image of thin section of Populus deltoides wood (F and V refer to fibre and vessel element, respectively) (Joseleau, Imai et al. 2004).

4.2 Chemical composition of wood and inner bark

4.2.1 Wet chemical analyses

Figures 31 and 32 summarize the overall chemical composition and carbohydrate composition of willow wood obtained from the four studied willow clones. The absolute contents of carbohydrates, lignin, extractives and ash were determined experimentally and the unanalysed difference was referred to as 'others'. These include for example the acetyl groups of xylan that are known to be present in high quantities in hardwood species (SPRINGER, ZOCH 1968; Chirat, Lachenal et al. 2012). The overall chemical composition was mostly in agreement with the previous report on desert willow (*Salix psammophila*, Spsa), albeit with some differences. The content of the extractives (2.8%) obtained from acetone extraction of willow wood was similar to the 3.1% reported for desert willow extracted with ethanol/benzene (1:2 v/v). The average lignin content of the studied willow species was higher than for desert willow, whereas the proportion of holocellulose was higher in desert willow (Kubo, Hashida et al. 2013). Similar work on *Salix viminalis* showed a comparable holocellulose content with the four willow clones studied whereas their ash content (0.5%) was much lower than reported for *Salix viminalis* (1.71%) (Lavoie, Capek-Menard et al. 2010). More detailed information on the chemical analyses are presented in Appendix 4.2.

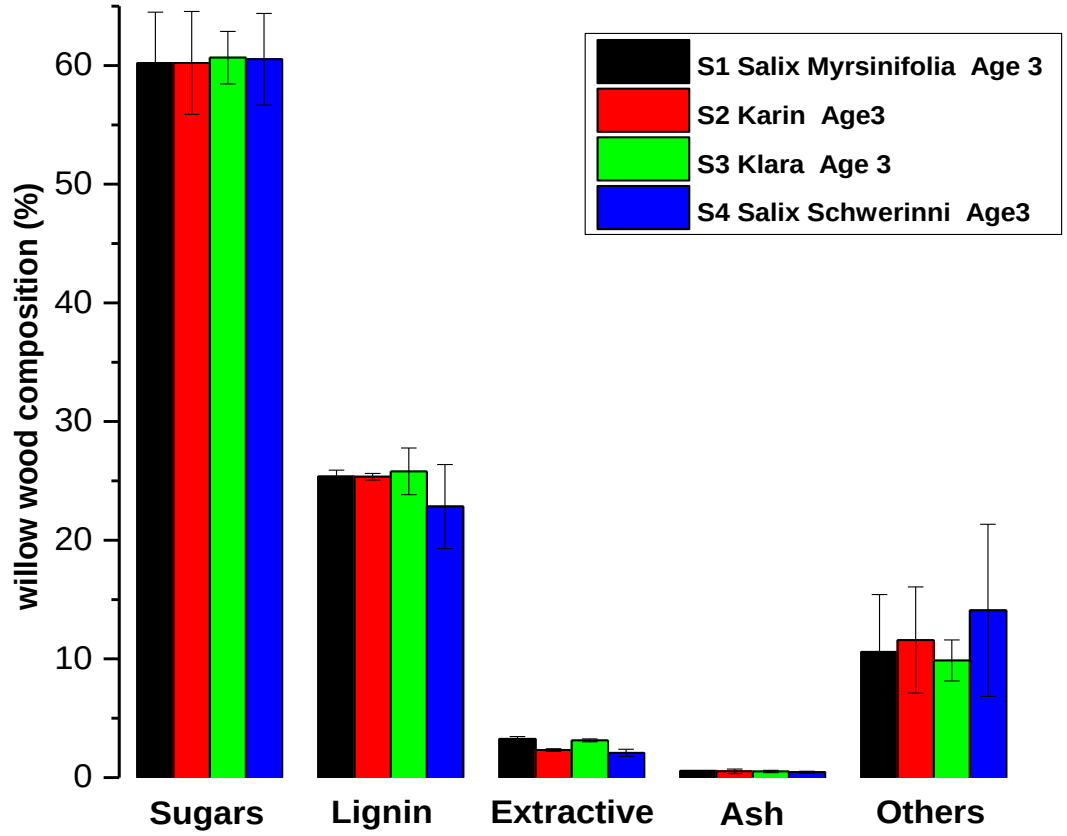

Figure 31. Chemical composition (% on original dry mass) of wood in willow clones.

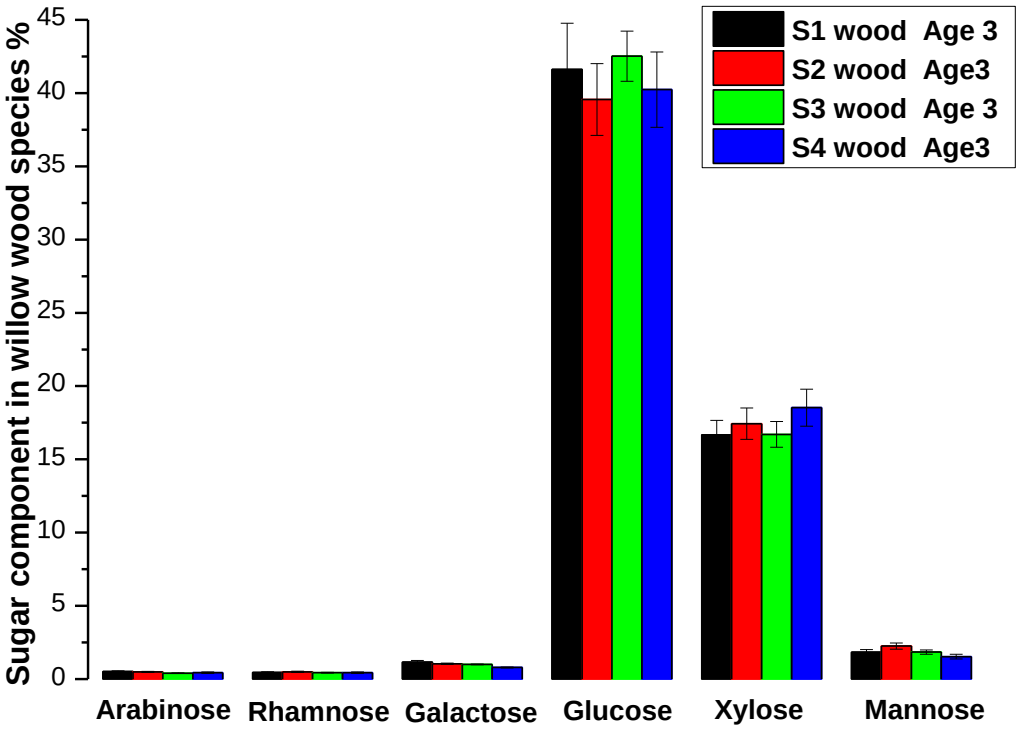

Figure 32. Carbohydrate composition (% of anhydrosugars on the original dry mass) of wood from willow clones.

Figures 33 and 34 show the chemical composition of the willow inner bark in the four willow hybrids. Relatively little reference literature is available on the chemical composition of inner bark in any hardwood

and softwood species. For example, Lebanon cedar's (*Cedrus libani*) inner bark showed a lower extractives content (10.69 %, extracted with ethanol-benzene) and higher Klason lignin content (28.29 %) in comparison with the chemical composition of willow inner bark (Usta, Kara 1997). Also spruce inner bark contained little extractives (13%, extracted by acetone) in comparison with willow inner bark. In addition the Klason lignin content of Norway spruce inner bark was lower (15%) than in willow inner bark (Krogell 2012). The differences in the lignin content between the species could possibly be explained by the varying functions of lignin in plants such as contribution to light and drought tolerance especially under the desert and mountain drought conditions (Hu, Li et al. 2009).

This is further confirmed by the chemical composition of the phloem and cork fractions of Turkey oak (*Quercus cerris*) (Sen, Miranda et al. 2010). Its inner bark has twice as high lignin content (35.4%) as willow inner bark has. On the contrary, the inner bark of Turkey oak has relatively low carbohydrate (30.6%) and hydrophilic extractives (6.5 %) contents. Figure 35B summarizes the differences in the chemical composition of the inner bark from Lebanon cedar, pine, Turkey oak and willow (Karin). More detailed information on the chemical composition is presented in Appendix 4.3.

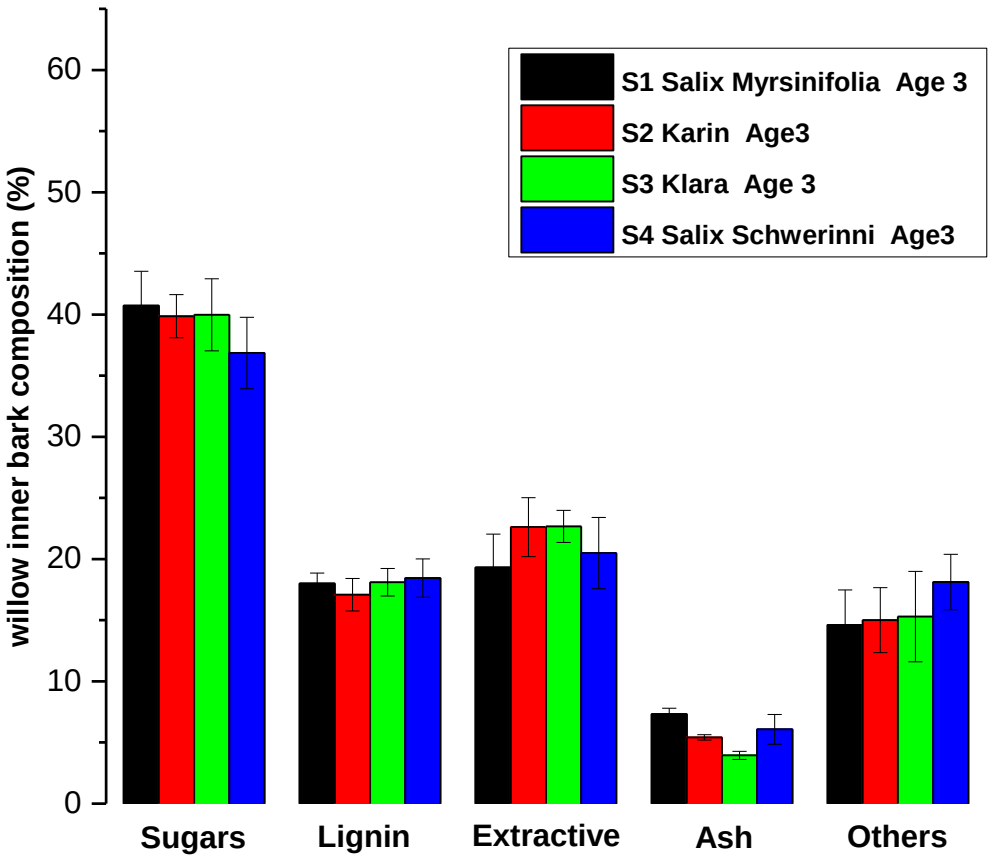

Figure 33. Chemical composition (% of the original dry mass) of inner bark of willow clones.

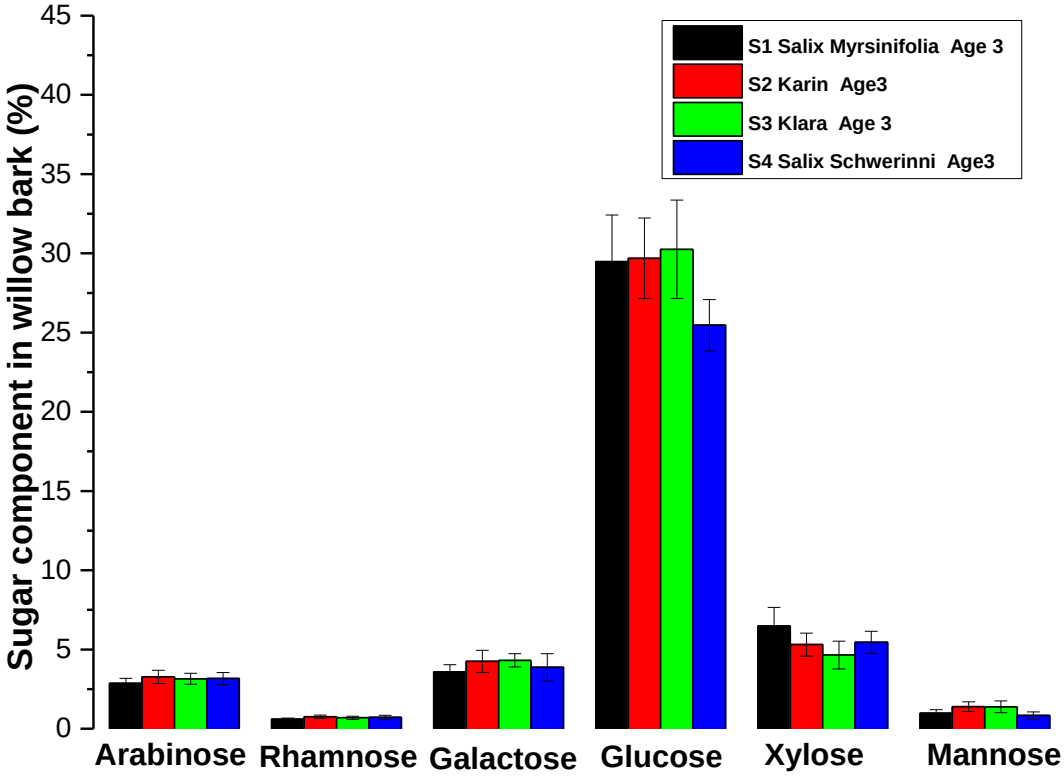

Figure 34. Carbohydrate composition (% of anhydrosugars on the original dry mass) of inner bark from willow clones.

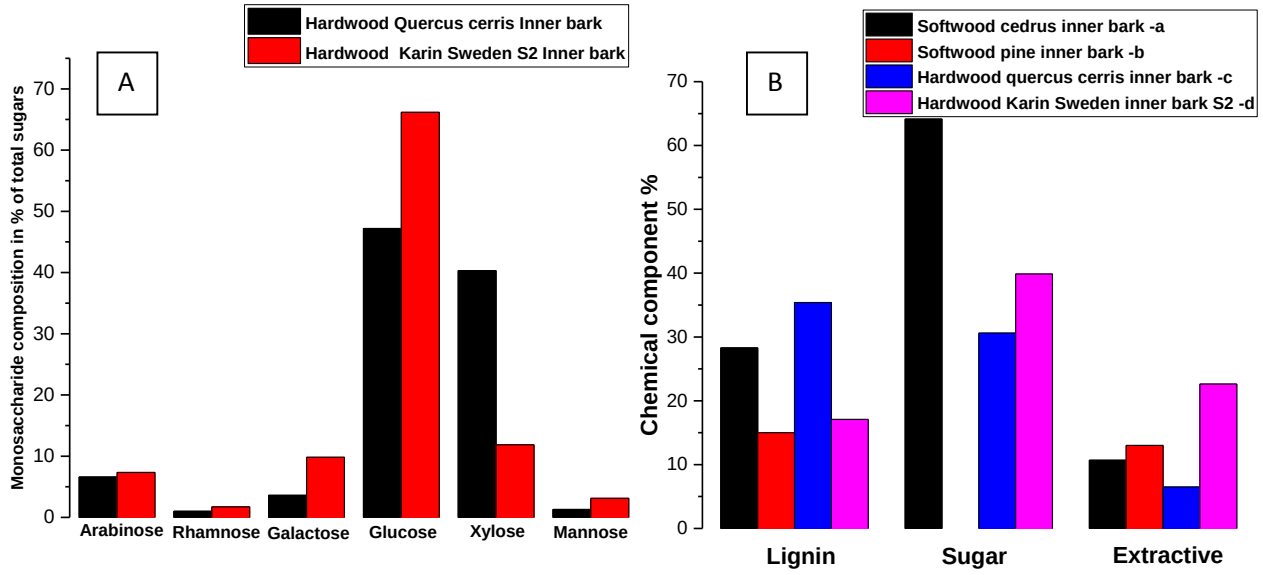

Figure 35. (A) Comparison of inner bark's monosaccharide composition between willow (Karin) and Turkey oak, (B) Chemical composition of inner bark in softwood (pine, Lebanon cedar) and hardwood species (Turkey oak, willow (Karin)). a) Ethanol-benzene extraction (Usta, Kara 1997), b) Aqueous acetone extraction (Krogell 2012), c) Extraction with dichloromethane, ethanol and water (Sen, Miranda et al. 2010), d) Acetone extraction.

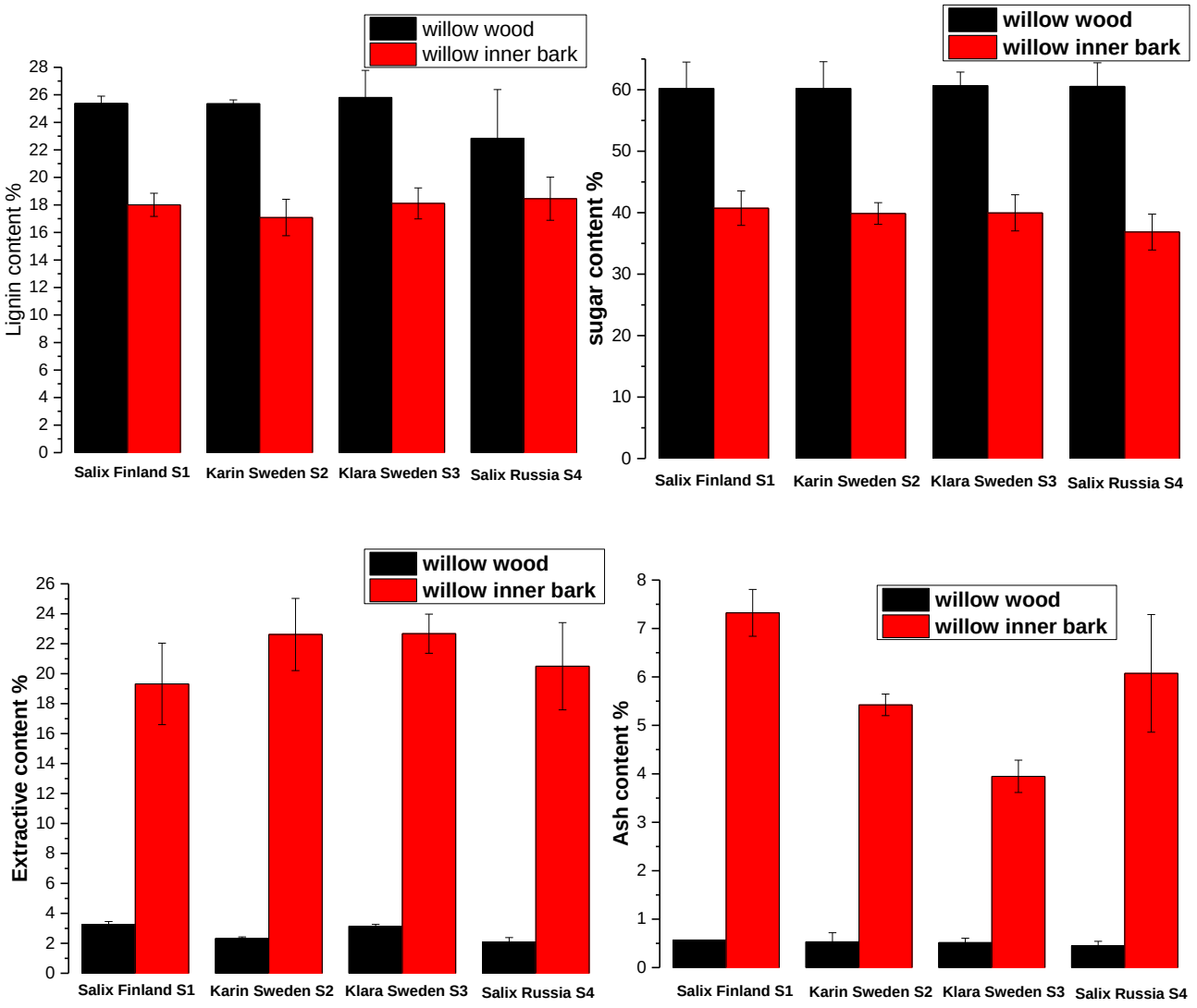

Figure 36. Comparison of chemical composition (% on original dry mass) between willow wood and inner bark of willow clones.

The acetone extract content of willow inner bark (19-23 %) was remarkably higher than the extractive content of willow wood (2-3 %). Also the ash content of the inner bark (4-7 %) was ten times higher than the ash content of the wood (0.5 %). On the contrary more carbohydrates and lignin were present in the wood than the inner bark of willow, mainly because of the high amounts of extractives and ash in the bark.

As shown in Figure 37, glucose was the main neutral monosaccharide in both the inner bark and wood while xylose was the dominating non-cellulosic sugar, indicating that xylan was the main hemicellulose although its proportion was much higher in wood. Arabinose, galactose and rhamnose were present in the inner bark in large amounts. These monosaccharides are characteristic components of pectins that thus form a significant amount of the polysaccharides in the inner bark. Galacturonic acid is another major component of pectin although the uronic acids were not quantified in this work. However, the higher content of 'others' in the inner bark support its high pectin content (FRY, Stephen 1982). The detailed monosaccharide composition results can be found in Appendix 4-2. Interestingly the xylose content of the inner bark of Turkey oak was very high while the contents of galactose and rhamnose were much lower than in the willow inner bark (Figure 35A). Thus the high pectin content is possibly a characteristic feature for willow inner bark. More information on the chemical composition can be found in Appendix 4.3.

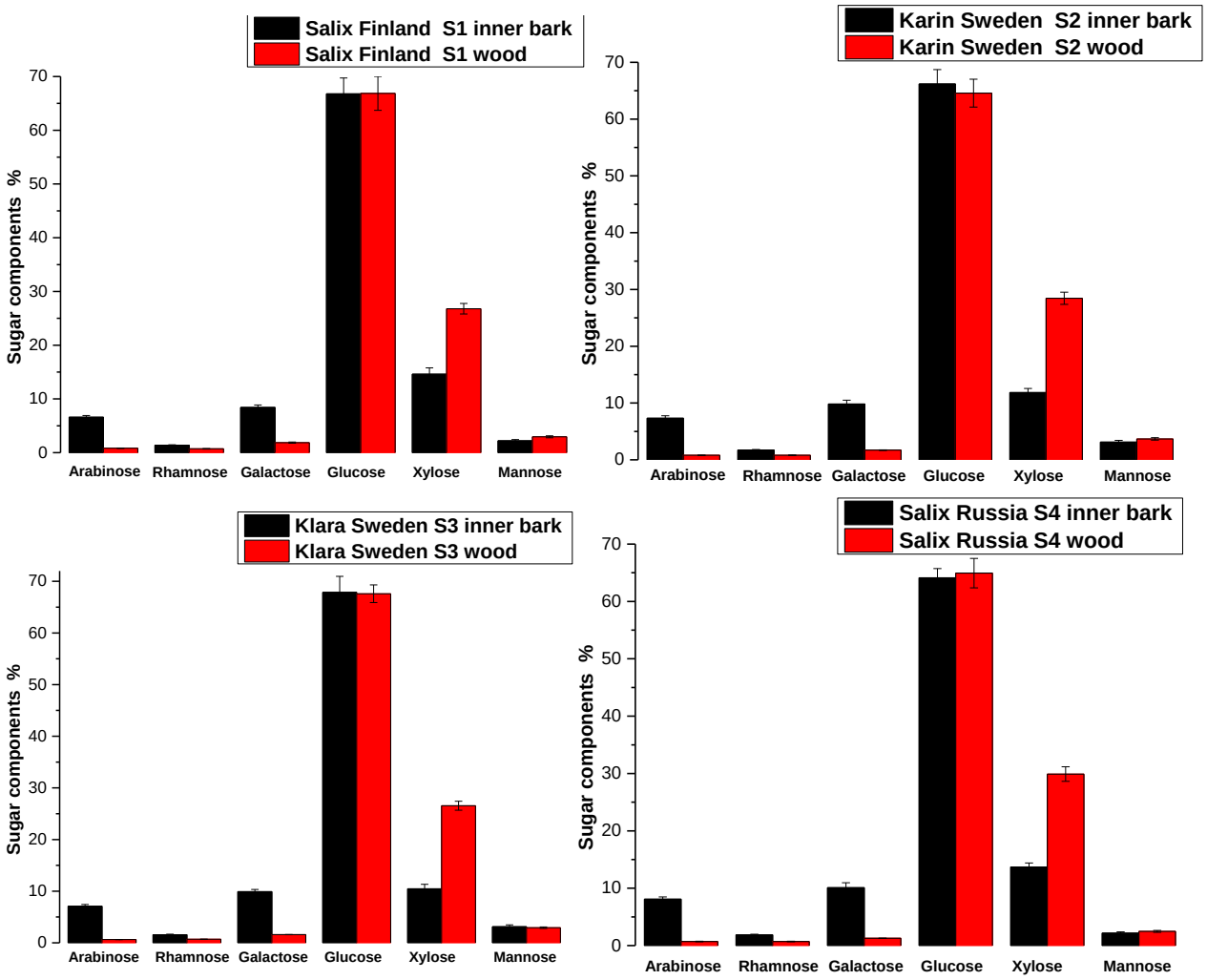

Figure 37. Comparison of the neutral sugar compositions between the wood and inner bark of willow clones.

Although the average chemical composition of the studied willow wood species were quite comparable to those of other hardwoods, there are considerable differences in the chemical composition between the inner bark and wood of the studied willow clones.

4.2.2 FTIR spectroscopy of extractives from inner bark

FT-IR spectra of hydrophilic extractives showed the characteristic bands at 2923-2856 cm^{-1} assigned to the methyl and methylene group (Figure 37). Additionally, a strong hydroxyl group absorption could be identified at the region of 3550- 3450 cm^{-1}. The presence of aromatic bonds could be detected near 1605 cm^{-1}. Furthermore, the band near 1691 cm^{-1} could be assigned for carbonyl group (e.g. acetophenone type). The smaller bands at 1640 and 1740 cm^{-1} could indicate the presence of conjugated aromatic structures (e.g. *p*-coumaryl alcohol type) and fats, respectively. Because salicin is often reported to be present in willow bark (Schmid, Kotter et al. 2001), the IR spectrum of salicin was also recorded (Figure 38). However, the characteristic bands of salicin were not recorded in the spectra of the acetone extracts. The IR spectra of all willow inner bark extracts are shown Appendix 5.

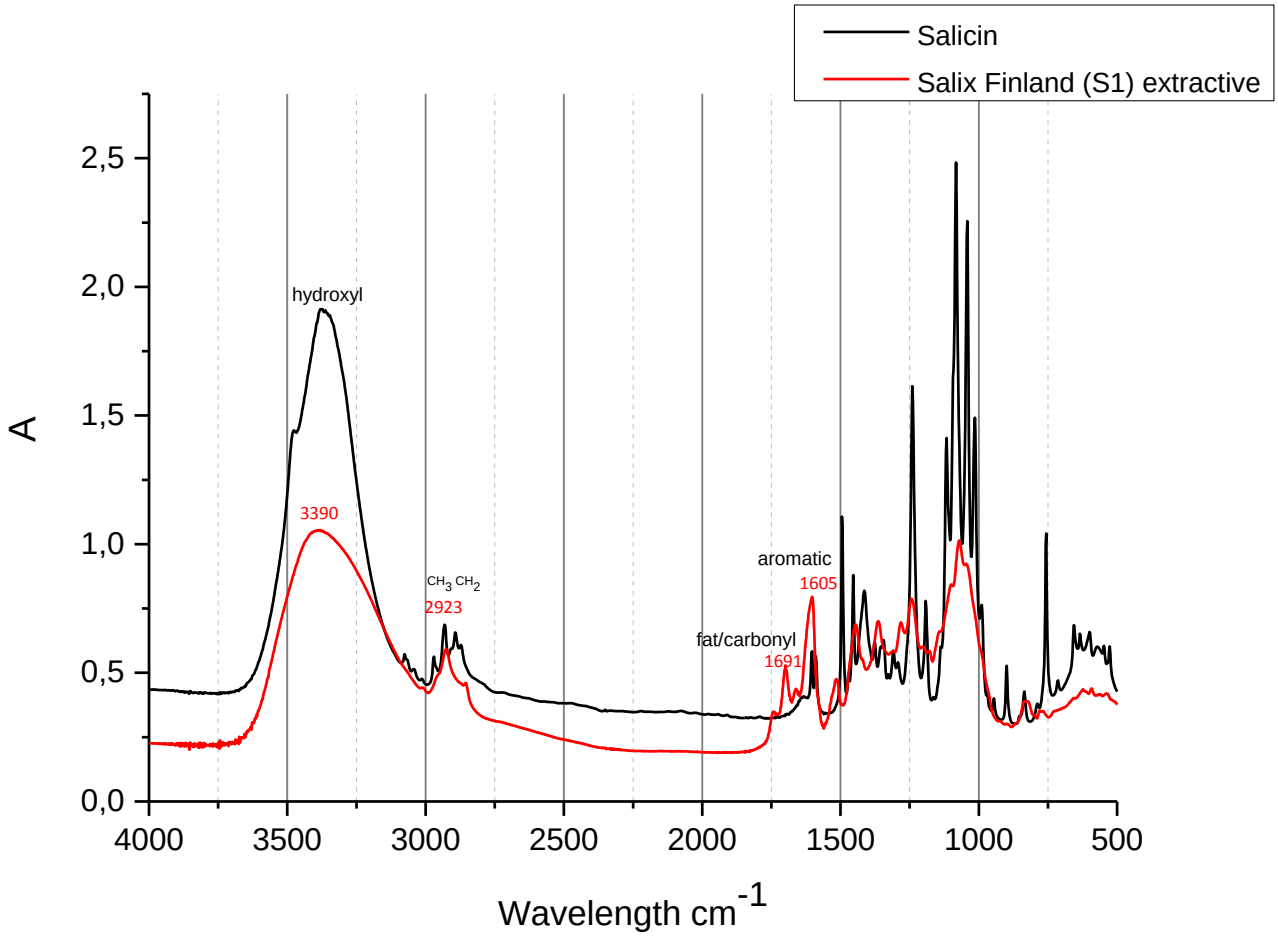

Figure 38. Infrared spectra of acetone extract of willow (S1 Finland) inner bark and pure salicin.

4.2.3 Raman microscopy and UV resonance Raman (UVRR)

Raman mapping of the willow inner bark (Salix Finland, S1) was performed by using a sample that was pretreated with acetic acid/hydrogen peroxide for 48 h at 40° C. This maceration treatment removed part of the lignin that causes fluorescence by the laser excitation at 532 nm. Figure 39 shows the spectra on particular locations and Raman images of the wax, pectin, fatty acids in tangential inner bark. The spectra shows a strong band near the region (1090 cm^{-1}) that assigned to cellulose, while Raman band located 854-860 cm^{-1} can be assigned to pectin from sclerenchyma (Gierlinger, Keplinger et al. 2012). The bands on the region of 1463- 1490 cm^{-1} are assigned to CH_2 groups present in lipids from waxes (Edwards, Falk 1997).

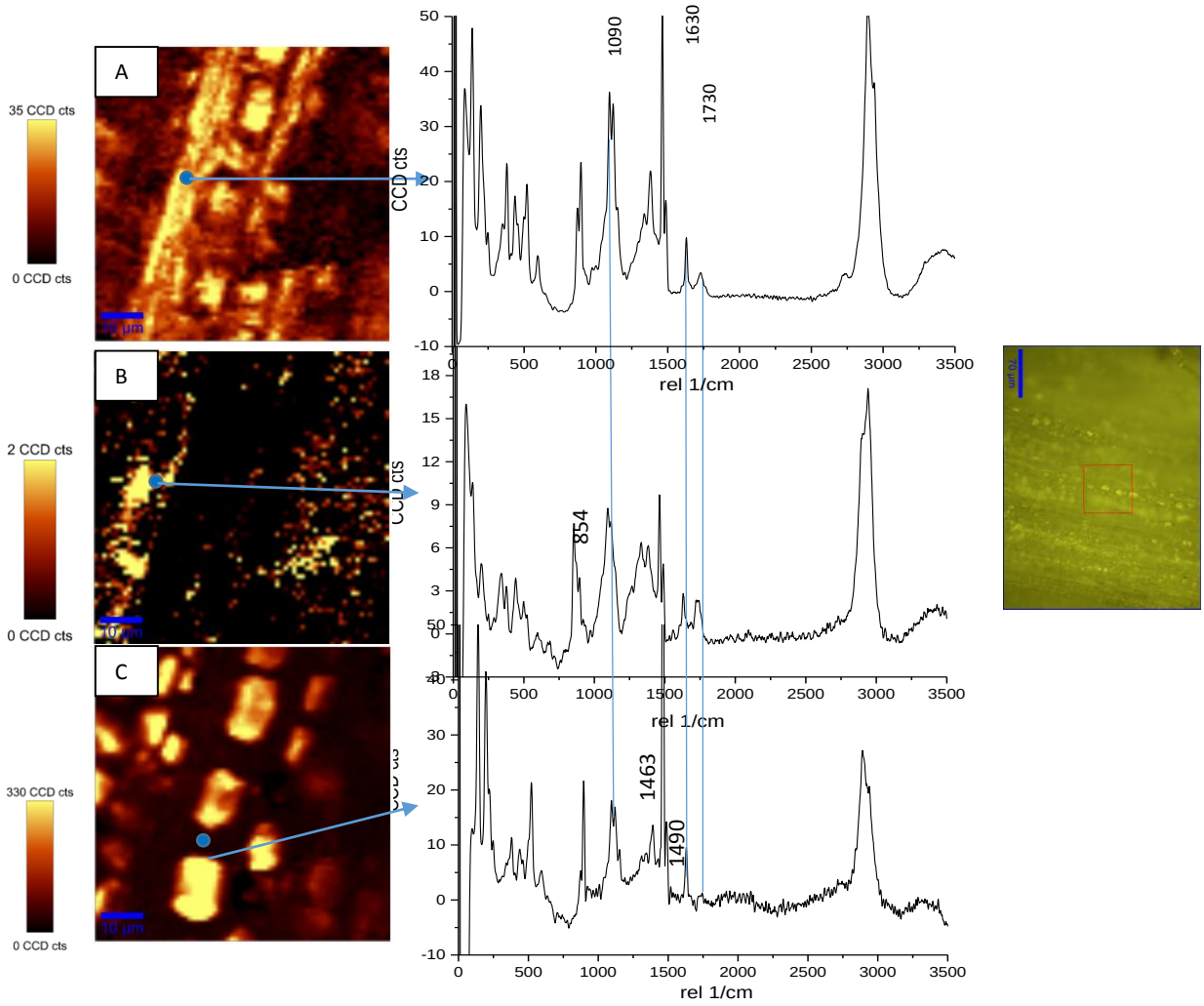

Figure 39. Raman images of willow inner bark (after maceration): (A) (1090-1100 cm^{-1}) band assigned to cellulose, (B) (854-860 cm^{-1}) to pectin, (C) (1450-1473 cm^{-1}) to fatty acids. All spectra were collected at the excitation wavelength of 532 nm.

Figure 40 shows the UVRR spectra of original willow inner bark (Karin), acetone extracted bark, the acetone extract and lignin isolated from the inner bark at the excitation wavelength of 244 nm. The band at 1600 cm^{-1} can be assigned to the symmetric aromatic ring stretching (Jaaskelainen, Toikka et al. 2009).The main conclusion from these spectra is that the acetone extract contains aromatic compounds that have additional Raman bands at 1372 and 1426 cm^{-1}.

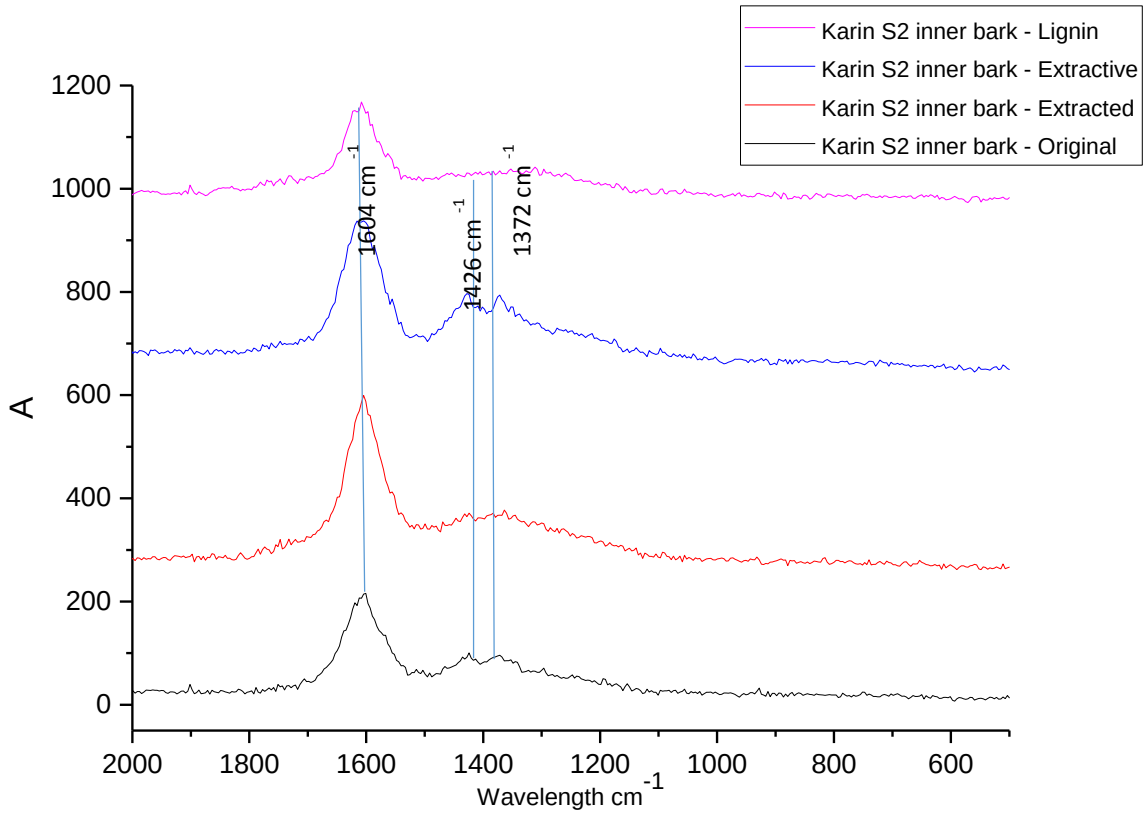

Figure 40. UV Raman spectra of willow inner bark, acetone extracted bark, the acetone extract and lignin isolated from inner bark. All spectra were collected at the excitation wavelength of 244 nm.

5 Conclusion

The aim of this study was to gain basic information on the chemistry and structure of willow inner bark for assessing the potential of the bark as a source of fibres and chemicals. A prestudy revealed that kraft cooking is suitable method to separate the fibres of willow inner bark. However, standard chlorite treatment (sodium chlorite and acetic acid) proved to be a suitable method to prepare the fibres for analytical purposes.

In general, the length and aspect ratio of the willow inner bark fibres are approximately twice higher than the corresponding dimensions of willow wood fibres although there was some variation between willow species. Clearly the sclerenchyma fibres of willow inner bark have potential as fibre source for special material uses such as composite applications.

Unique features of the willow inner bark fibres were revealed by SEM and TEM imaging. The fibres form lignin bound bundles of tens of individual fibres that have very small lumina. Lignin distribution between the fibres and within individual cell wall layers can be identified by $KMnO_4$ staining and TEM. The most intensely stained areas are the cell corner and middle lamellae. A varying number of layers (3 to 8) are present in the sclerenchyma fibres which indicates a different lignin distribution in comparison with the normal wood cell wall. The number of concentric sclerenchyma fibre bundle layers, observable by SEM or optical microscopy, indicates the age of the willow species.

The willow inner bark contains approximately 8 times more hydrophilic extractives (extractable with acetone) than the corresponding wood section (19-23% and 2-3%, respectively). The inner bark and wood fractions show similar average degree of lignification (similar lignin-to-carbohydrates ratio) although the lignin content (18 % vs. 24 % in wood) of the inner bark is lower due to its higher extractives and ash contents. For the same reason the polysaccharide content of willow wood is approximately 1.5 times the polysaccharide content in the inner bark section (60% and 40% for wood and inner bark respectively). In addition, the arabinose and galactose content in the willow inner bark is approximately twice higher than in wood due to the presence of pectin, whereas more cellulose and xylan is present in wood than the inner bark.

Due to the higher abundance of ash and low sintering point of inner bark (lower than that of wood ash), the combustion of inner bark can lead to fouling which would damage the combustors. As a result, inner bark is not an ideal fuel for direct energy production. It is also not a particularly promising feedstock for secondary fuel production. Willow's inner bark comprises a larger fraction of extractives and lignin than the wood section (around 40 wt. % and 20 wt. % for willow inner bark and wood respectively on a dry basis). The aromatic compounds from the inner bark may retard both the hydrolysis and fermentation reactions necessary for ethanol production.

A net advantage of the willow would be its fast growth as well as a high biomass production. The utilization of the inner bark for chemicals and fibre materials through its fractionation could be more feasible and economically advantageous than the direct energy use of the whole willow biomass.

Even though the analytical chlorite treatment approach for fibre separation is not sufficient to justify as an industrial pulping process, the chemical composition and the mechanical properties of the inner bark fibres as an added value co-product in a biorefinery process that aims at biofuels and/or green chemicals production from debarked willow wood has merits and could possibly be feasible.

6 Future research

Because the extractives content and composition of willow bark varies depending on the season, this annual variation should be studied carefully to identify the best time for harvesting the crop. The extractives composition should be studied by GC-MS to identify the commercially most potential chemical compounds. Different polar and nonpolar solvents could be used for quantifying the lipophilic and hydrophilic extractives more systematically. The amount and true chemical character of lignin is also not yet fully elucidated. Structural analysis of the lignin in the inner bark by NMR spectroscopy could help in identifying an ideal treatment for fibre separation. Chemical mapping of the inner bark's cross section by Raman microscopy could serve the same purpose. The pectic polysaccharides are also of potential interest and should be studied further.

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Appendix

Contents

1 Fibre basic properties

1.1 Four willow clones dyed fibres under optical microscopy

Figure 1-1-1. S2 hybrid 'Karin' from 1 year - 4 years old (imaged by Holopainen-Mantila Ulla, VTT).

Figure 1-1-2. Aging of S2 hybrid 'Karin' from 1 year - 4 years old (imaged by Holopainen-Mantila Ulla, VTT).

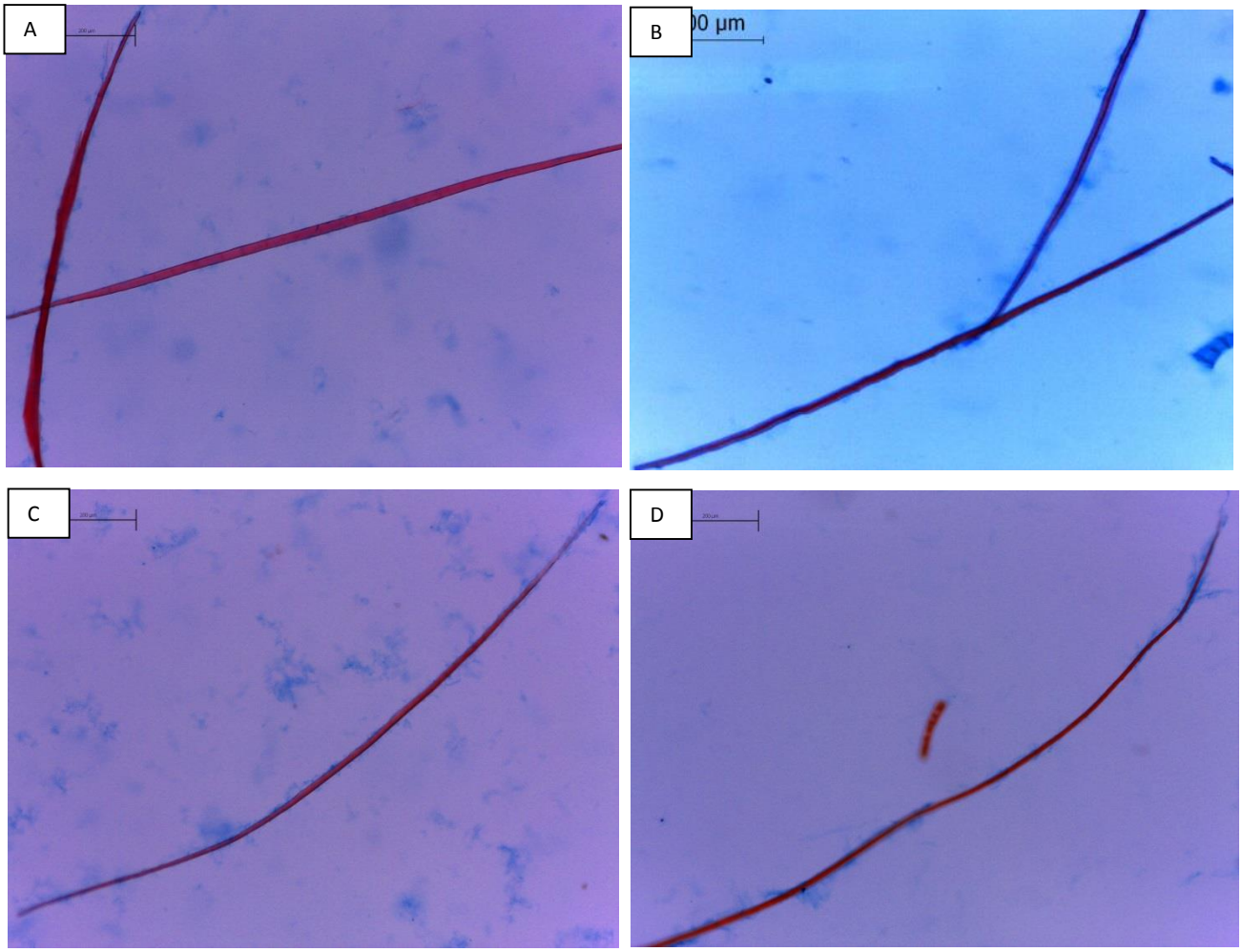

Figure 1-1-3. Four studied willow clones dyed fibres after chlorite treatment under optical microscopy: (A) S1 Salix Myrsinofolia (Finland), (B) S2 Karin (Sweden), (C) S3 Klara (Sweden), (D) S4 Salix schwerinii (Russia).

1.2 Calculation and analytical method (curl /kink / fibre length/ fibre width)

Fiber curl $\mathrm{Li} = \left(\frac{\mathrm{Lc}}{\mathrm{Lp}} - 1\right) * 100\%$

Where

Curl Li = curl of fibre

Lc = true length of fibre (along the center line)

Lp = projected length of fibre (linear measurement)

Kink $= \frac{(\mathrm{n1}+2\times\mathrm{n2}+3\times\mathrm{n3}+4\times\mathrm{n4})}{\mathrm{Lc}}\ [1/\mathrm{mm}]$

Where

n1= number of 10-20° kinks in fibre

n2 = number of 21-45° kinks in fibre

n3 = number of 46-90° kinks in fibre

n4 = number of over 90° kinks in fibre

Lc = centerline length of fibre (mm)

Arithmetic average fibre length $L\ (n) = \frac{\sum(ni*l)}{\sum ni}\ [mm]$

Length-weighted fibre length $L\ (l) = \frac{\sum(ni*Li)}{\sum ni\ Li}$ [mm]

Weight-weighted average fibre length $L\ (w) = \frac{\sum(ni*Fi)}{\sum(ni\ .Fi)}$ [mm]

Average fibre width $Aw = \frac{\sum(ni*wi)}{\sum ni}$ [um]

1.3 Recipe for chlorite treatment and fibre basic properties

Table 1-3-1. Recipe for chlorite treatment for getting fibres (willow wood and inner bark).

Willow Wood	Willow wood o.d (g)	Distilled water (ml)	99,8 % acetic acid (ml)	Sodium chlorite (g)	Temperature (°C)	Reaction time (H)	Swing frequency (min^{-1})
Salix Finland S1	2.51	80	0.5	1.577/1.502/1.607/1.476/1.472	80	5	85
Karin Sweden S2	2.53	80	0.5	1.543/1.546/1.546/1.617/1.575	80	5	85
Klara Sweden S3	2.45	80	0.5	1.463/1.506/1.543/1.502/1.484	80	5	85
Salix Russia S4	2.50	80	0.5	1.577/1.489/1.482/1.484/1.609	80	5	85

Willow Inner bark	willow inner bark o.d (g)	Distilled water (ml)	99,8 % acetic acid (ml)	Sodium chlorite (g)	Temperature (°C)	Reaction time (H)	Swing frequency (min^{-1})
Salix Finland S1	2.49	80	0.5	1.455/1.551/1.563/1.555/1.455	80	5	85
Karin Sweden S2	2.49	80	0.5	1.511/1.501/1.553/1.503/1.528	80	5	85
Klara Sweden S3	2.49	80	0.5	1.560/1.559/1.554/1.492/1.581	80	5	85
Salix Russia S4	2.49	80	0.5	1.553/1.501/1.544/1.562/1.524	80	5	85

Table 1-3-2. Fibre properties based on the chlorite treatment (willow wood and inner bark).

Wooden Sample /Age 3	Fibre length (mm)	L (n) (mm)	L (w) (mm)	Length measured (pcs)	Fibre width (µm)	Width measured (pcs)	Aspect ratio
S1 Salix Finland	0.56	0.42	0.80	21775	19.05	17953	29
S2 Karin Sweden	0.54	0.42	0.74	21557	19.30	18444	28
S3 Klara Sweden	0.63	0.43	1.00	17543	22.20	14279	28
S4 Salix Russia	0.49	0.37	0.66	19562	18.20	15617	27

Wooden Sample/ Age 3	Fibre curl (%)	Curl measured (pcs)	Fibre kink (l) (1/m)	Kink (l)measured (pcs)
S1 Salix Finland	0.02	17953	46.27	30246
S2 Karin Sweden	0.02	18444	57.55	28249
S3 Klara Sweden	0.02	14279	51.16	22967
S4 Salix Russia	0.02	15617	41.64	23863

Willow inner bark/ Age 3	Fibre length (mm)	L (n) (mm)	L (w) (mm)	Length measured (pcs)	Fibre width (µm)	Width measured (pcs)	Aspect ratio
S1 Salix Finland	1.50	0.79	2.16	14858	23.7	9665	63
S2 Karin Sweden	1.71	1.04	2.52	8908	24.1	8035	71
S3 Klara Sweden	1.60	1.02	2.52	8864	23.0	8116	70
S4 Salix Russia	1.19	0.65	1.75	12874	23.6	8633	50

Inner bark Sample /Age 3	Fibre curl (%)	Curl measured (pcs)	Fibre kink (l) (1/m)	Kink (l)measured (pcs)
S1 Salix Finland	0.08	9665	277.37	29153
S2 Karin Sweden	0.10	8035	328.16	15597
S3 Klara Sweden	0.09	8116	329.68	17846
S4 Salix Russia	0.07	8633	274.83	23097

2 TEM image of fibre wall

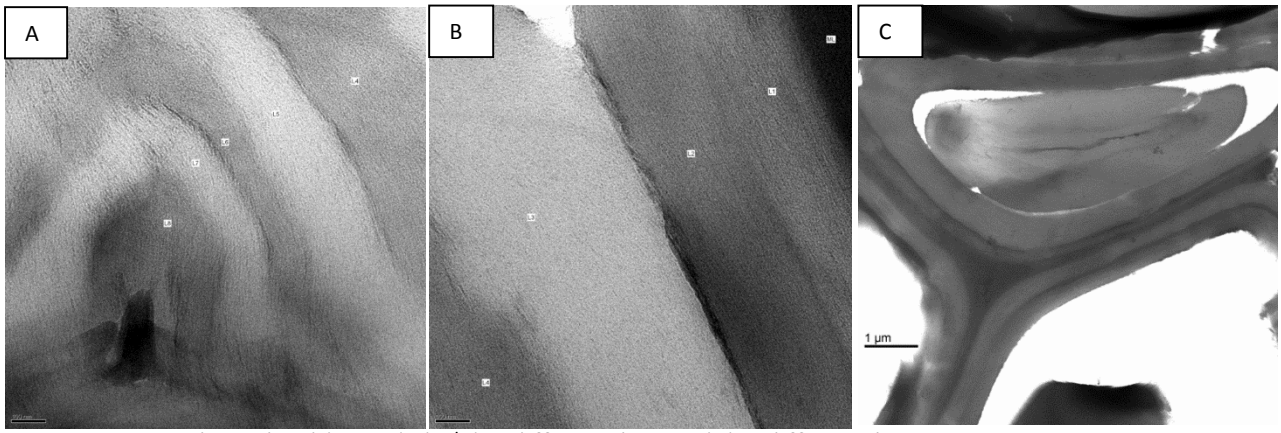

Figure 2-1. S1 Salix Finland (Age 3): (A/B) 8 different layers, (C) 5 different layers.

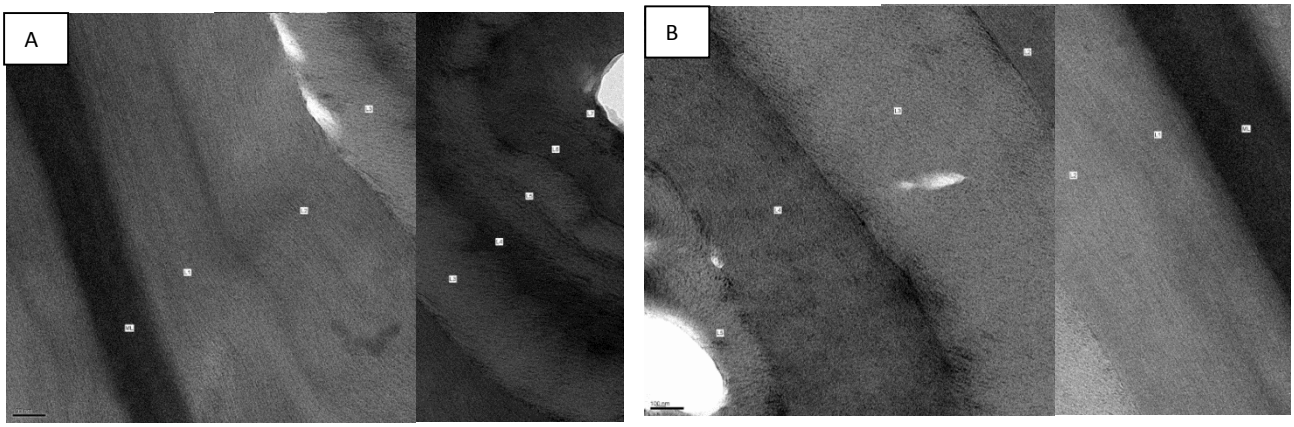

Figure 2-2. S2 Karin Sweden (Age 3): (A) 7 different layers, (B) 5 different layers.

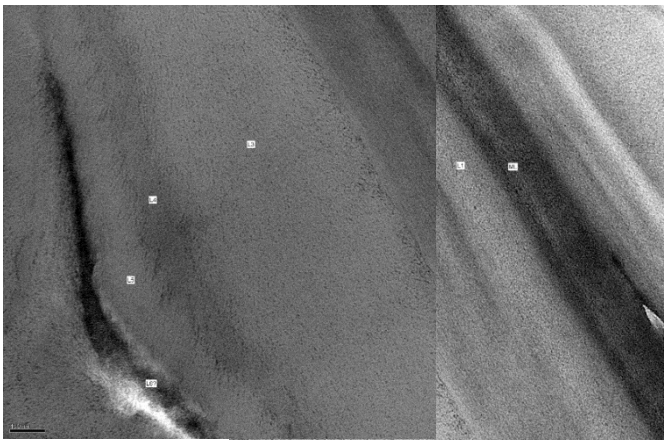

Figure 2-3. S3 Klara Sweden (Age 3): 5 different layers.

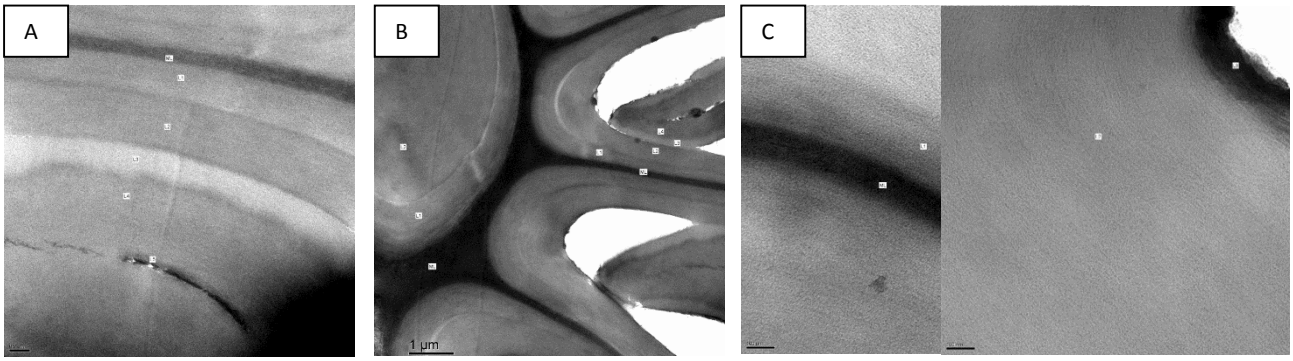

Figure 2-4. S4 Salix Russia (Age 3): (A-C) L2/L3/L4/L5.

3 SEM image of cell structure

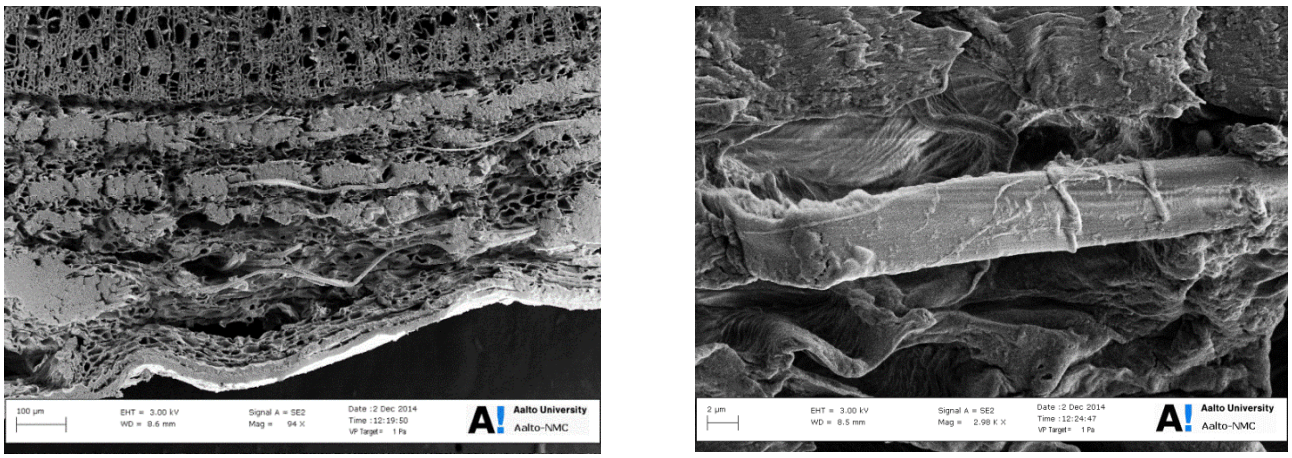

Figure 3-1. S1 Salix Finland (Age 3) bundles of sclerenchyma fibres.

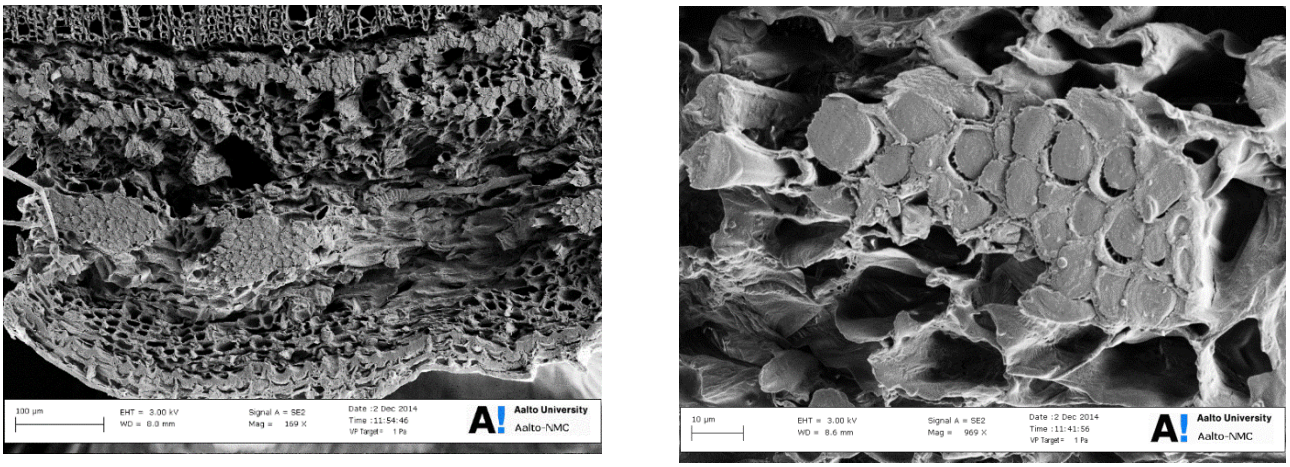

Figure 3-2. *S2* Karin Sweden (Age 3) bundles of sclerenchyma fibres.

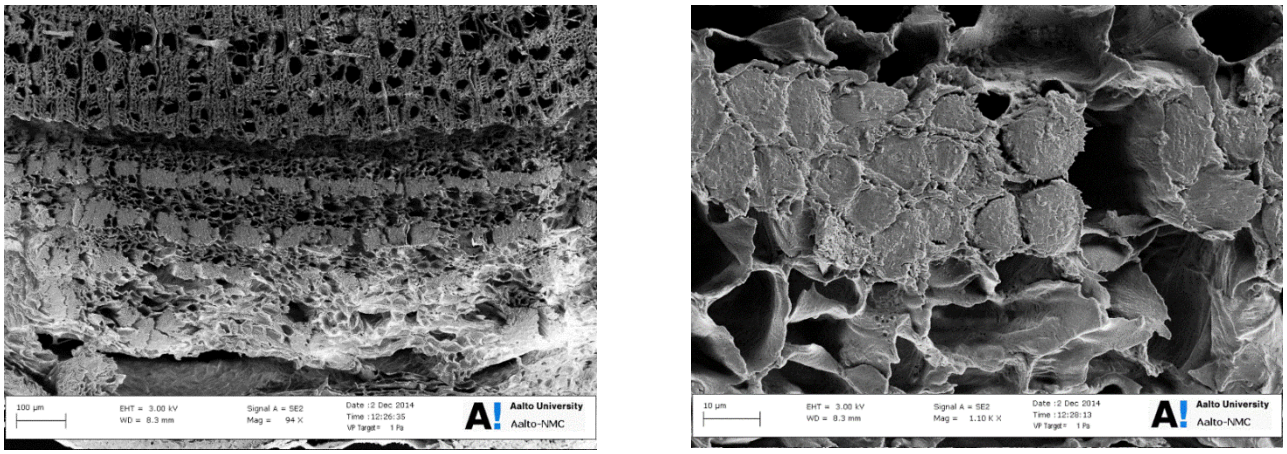

Figure 3-3. S3 Klara Sweden (Age 3) bundles of sclerenchyma fibres.

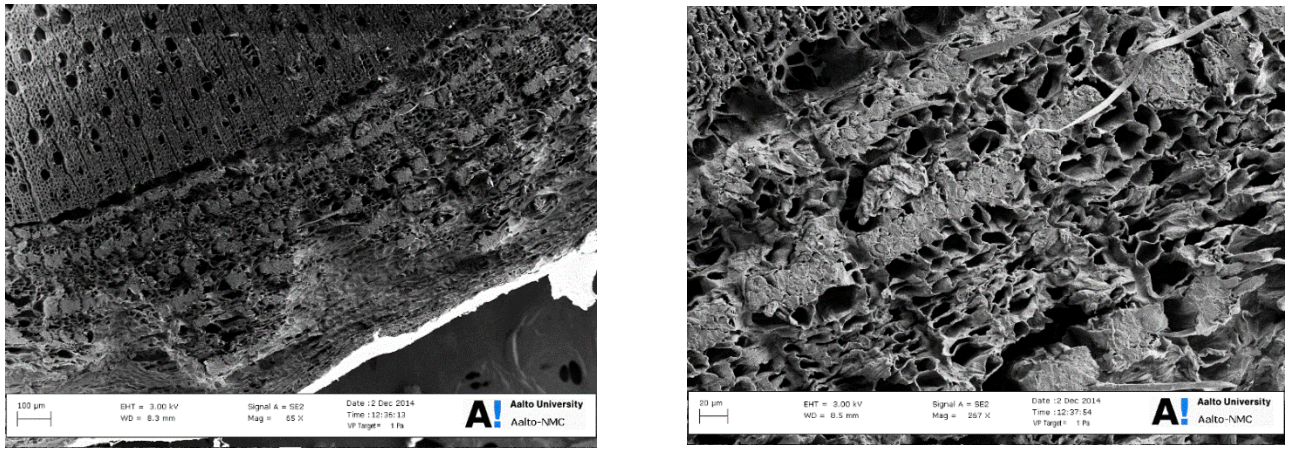

Figure 3-4. S4 Salix Russia (Age 3) bundles of sclerenchyma fibres.

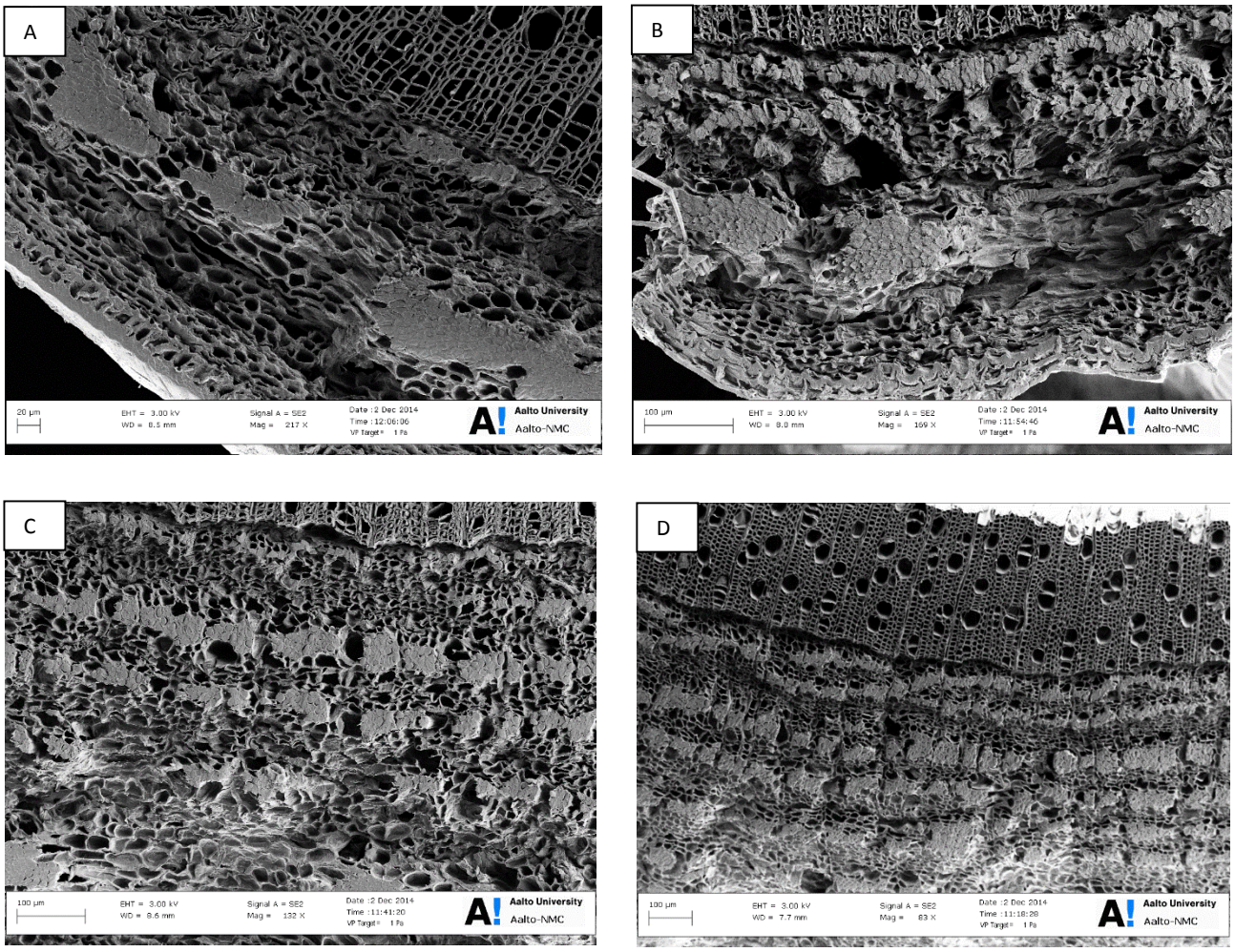

Figure 3-5. SEM image of S2 hybrid 'Karin' from 1 year - 4 years old: (A) 1 year old, (B) 2 year old, (C) 3 year old, (D) 4 year old.

4 Chemical analysis

4.1 Ash determination

Table 4-1-1. Four sets of data for ash determination-Willow inner bark (20141128WB/ 20141210WB).

20141128WB	Container (g)	Dry matter	O.D sample (g)	Container + sample (before) (g)	After (g)	Ash content
S1	16.34	0.92	1.11	17.55	16.44	0.08
S2	14.03	0.93	1.50	15.65	14.12	0.06
S3	17.20	0.91	1.62	18.97	17.27	0.04
S4	18.65	0.98	2.03	20.72	18.79	0.07

201412010WB	Container (g)	Dry matter	O.D sample (g)	Container + sample (before) (g)	After (g)	Ash content
S1	17.87	0.9322	0.80	18.73	15.54	0.07
S2	15.45	0.9254	1.07	16.60	17.92	0.05
S3	15.48	0.9281	1.03	16.58	15.52	0.04
S4	13.67	0.9338	1.06	14.80	13.75	0.07

20141128WB	Container (g)	Dry matter	O.D sample (g)	Container + sample (before) (g)	After (g)	Ash content
S1	18.64	0.94	1.09	19.80	18.72	0.07
S1	18.39	0.94	1.25	19.72	18.48	0.07
S2	18.02	0.94	1.26	19.36	18.08	0.04
S3	18.00	0.94	0.79	18.84	18.03	0.03
S4	18.59	0.94	0.95	19.61	18.65	0.06
S4	17.96	0.94	0.69	18.70	18.01	0.06

20141128WB	Container (g)	Dry matter	O.D sample (g)	Container + sample (before) (g)	After (g)	Ash content
S1	18.48	0.92	1.56	20.17	18.62	0.08
S2	17.58	0.93	1.91	19.64	17.69	0.05
S3	18.45	0.91	1.46	20.05	18.52	0.04
S4	20.16	0.98	1.77	21.96	20.28	0.07

Table 4-1-2. Three sets of data for ash determination - Willow wood (20150109WW/20141128WW).

20150109WW	Container (g)	Dry matter	O.D sample (g)	Container + sample (before) (g)	After (g)	Ash content
S1	19.84	0.96	1.75	21.67	19.85	0.01
S2	14.63	0.96	1.90	16.60	14.63	0.00
S3	17.31	0.97	2.10	19.49	17.32	0.01
S4	17.42	0.96	2.25	19.75	17.43	0.01

20141128WW	Container (g)	Dry matter	O.D sample (g)	Container + sample (before) (g)	After (g)	Ash content
S1	15.12	0.96	1.72	16.92	15.13	0.01
S1	18.09	0.96	1.84	20.01	18.10	0.01
S2	14.94	0.96	1.72	16.73	14.95	0.01
S2	18.49	0.96	1.68	20.24	18.50	0.01
S3	14.46	0.97	1.18	15.68	14.46	0.00
S3	17.49	0.97	1.81	19.36	17.50	0.00
S4	13.43	0.96	1.63	15.12	13.44	0.00
S4	13.10	0.96	2.75	15.96	13.12	0.00

20141128WW	Container (g)	Dry matter	O.D sample (g)	Container + sample (before) (g)	After (g)	Ash content
S1	9.35	0.96	0.62	10.00	9.36	0.01
S2	15.04	0.96	1.15	16.24	15.05	0.01
S3	14.99	0.97	1.44	16.48	15.00	0.00
S4	14.76	0.96	1.33	16.14	14.77	0.00

Table 4-1-3. Average value of the ash components.

	Ash inner bark (%)	STD willow inner bark	Ash wood (%)	STD willow wood
Salix Finland S1	7.32	0.48	0.57	0.00
Karin Sweden S2	5.42	0.22	0.53	0.19
Klara Sweden S3	3.95	0.33	0.51	0.09
Salix Russia S4	6.07	1.21	0.45	0.09

4.2 Ten sets of data recipe for chemical analysis (HPAEC)

Code name: 20141113WB Sample: four studied willow inner bark (**in % of total original mass**)

S1-Salix Finland inner bark; S2- Karin Sweden inner bark; S3- Klara Sweden inner bark; S4 - Salix Russia inner bark

No.1 Extractive content	**Aluminium (g)**	**Aluminium + dry sam (g)**	**Extractive (g)**	**O.D. original mass (g)**	**Extractive content**
S1	1.86	2.90	1.04	5.92	0.18
S2	1.85	3.12	1.27	5.45	0.23
S3	1.86	3.18	1.32	5.56	0.24
S4	1.86	2.61	0.75	3.61	0.21

No.2 Klason lignin	Amount (mg)	OD (mg)	Crucible (g)	Crucible + Lignin (g)	Klason lignin (mg)	Klason (%)
S1-1	338.80	317.10	29.70	29.76	62.50	19.71
S1-2	345.10	321.02	29.71	29.78	63.20	19.69
S2-1	300.40	277.25	29.70	29.75	50.70	18.29
S2-2	314.00	290.03	29.66	29.72	55.50	19.14
S3-1	345.70	320.24	29.63	29.70	70.30	21.95
S3-2	341.50	316.89	29.67	29.75	72.00	22.72
S4-1	325.10	299.68	29.71	29.77	61.60	20.56
S4-2	323.30	297.52	29.72	29.78	58.20	19.56

No.3 ASL	V. (ml)	Dilution	Absorbance. 205 nm	ASL (%)
S1-1	86.73	25.00	0.50	3.13
S1-2	86.73	25.00	0.49	2.99
S2-1	86.73	25.00	0.39	2.79
S2-2	86.73	25.00	0.40	2.73
S3-1	86.73	25.00	0.36	2.19
S3-2	86.73	25.00	0.38	2.36
S4-1	86.73	25.00	0.50	3.29
S4-2	86.73	25.00	0.48	3.16

No.4 SRS	Known c (mg/l)	Measured c (mg/l)	Standard correction
Arabinose	105.18	92.60	0.88
Rhamnose	105.37	96.92	0.92
Galactose	203.72	175.44	0.86
Glucose	1002.92	925.63	0.92
Xylose	499.54	424.45	0.85
Mannose	97.38	66.13	0.68

No.5 Chemical components	**Total sugars**	**Lignin**	**Extractive**	**Ash/ Acetate**	**Others**
Salix Finland inner bark S1	0.428	0.188	0.176	0.073	0.136
Karin Sweden inner bark S2	0.429	0.165	0.232	0.054	0.120
Klara Sweden inner bark S3	0.431	0.188	0.237	0.039	0.104
Salix Russia inner bark S4	0.407	0.184	0.209	0.061	0.139

No.6-1 HPAEC- S1	measured c	measure 20 - a	measure 20 - b	correction ratio	C(anhydro)	sugar	OD sample (mg)
Arabinose	128.28	128.28	128.28	0.88	128.22	0.04	317.10
Rhamnose	28.25	28.25	28.25	0.90	27.64	0.01	317.10
Galactose	158.77	158.77	158.77	0.90	165.92	0.05	317.10
Glucose	1245.83	1245.83	1245.83	0.90	1214.87	0.33	317.10
Xylose	267.93	267.93	267.93	0.88	277.49	0.08	317.10
Mannose	40.46	40.46	40.46	0.90	53.62	0.01	317.10
					Total	51.09 %	

No.6-2 HPAEC – S2	measured c	measure 22 - a	measure 22 - b	correction ratio	C(anhydro)	sugar	OD sample (mg)
Arabinose	138.36	138.36	138.36	0.88	138.30	0.04	277.25
Rhamnose	33.57	33.57	33.57	0.90	32.84	0.01	277.25
Galactose	163.74	163.74	163.74	0.90	171.12	0.05	277.25
Glucose	1166.27	1166.27	1166.27	0.90	1137.29	0.36	277.25
Xylose	218.80	218.80	218.80	0.88	226.61	0.07	277.25
Mannose	54.06	54.06	54.06	0.90	71.65	0.02	277.25
					Total	56%	

No.6-3 HPAEC – S3	measured c	measure 24 - a	measure 24 - b	correction ratio	C(anhydro)	sugar	OD sample (mg)
Arabinose	149.63	149.63	149.63	0.88	149.56	0.04	320.24
Rhamnose	34.06	34.06	34.06	0.90	33.32	0.01	320.24
Galactose	184.20	184.20	184.20	0.90	192.50	0.05	320.24
Glucose	1407.04	1407.04	1407.04	0.90	1372.08	0.37	320.24
Xylose	209.65	209.65	209.65	0.88	217.13	0.06	320.24
Mannose	66.43	66.43	66.43	0.90	88.04	0.02	320.24
					Total	56%	

No.6-4 HPAEC – S4	measured c	measure 26 - a	measure 26 - b	correction ratio	C(anhydro)	sugar	OD sample (mg)
Arabinose	153.25	153.25	153.25	0.88	153.18	0.04	299.68
Rhamnose	36.91	36.91	36.91	0.90	36.11	0.01	299.68
Galactose	169.64	169.64	169.64	0.90	177.28	0.05	299.68
Glucose	1171.70	1171.70	1171.70	0.90	1142.59	0.33	299.68
Xylose	250.85	250.85	250.85	0.88	259.80	0.08	299.68
Mannose	34.63	34.63	34.63	0.90	45.90	0.01	299.68
					Total	53%	

No. 7 Specific sugar	Salix Finland inner bark S1	Karin Sweden inner bark S2	Klara Sweden inner bark S3	Salix Russia bark S4
Arabinose	3.51	4.42	4.18	4.41
Rhamnose	0.77	1.06	0.93	1.04
Galactose	4.53	5.49	5.33	5.03
Glucose	33.83	35.64	37.81	32.31
Xylose	7.76	7.02	5.91	7.31
Mannose	1.52	2.27	2.37	1.41

ASL= Acid soluble lignin SRS= sugar recovery standard

Code name: 20141201WB Sample: four studied willow inner bark

S1-Salix Finland inner bark; S2- Karin Sweden inner bark; S3- Klara Sweden inner bark; S4 - Salix Russia inner bark

No.1 Extractive content	Aluminium (g)	Aluminium + dry sam (g)	Extractive (g)	O.D. original mass (g)	Extractive content
S1	1.86	2.80	0.94	5.21	0.18
S2	1.85	3.15	1.30	5.67	0.23
S3	1.86	3.52	1.66	7.75	0.21
S4	1.86	2.52	0.67	3.43	0.19

No.2 Klason lignin	Amount (mg)	OD (mg)	Crucible (g)	Crucible + Lignin (g)	Klason lignin (mg)	Klason (%)
S1-1	348.8	324.6	29.3	29.4	64.0	19.7
S1-2	317.0	295.6	19.8	19.8	61.0	20.6
S2-1	335.2	309.5	23.5	23.5	64.1	20.7
S2-2	325.3	300.9	29.2	29.3	59.5	19.8
S3-1	334.0	307.8	29.5	29.6	65.9	21.4
S3-2	317.5	292.5	29.3	29.3	63.6	21.7
S4-1	335.4	308.9	20.0	20.1	65.6	21.2
S4-2	340.0	316.0	29.6	29.7	65.5	20.7

No.3 ASL	V. (ml)	Dilution	Absorbance. 205 nm	ASL (%)
S1-1	86.73	25.00	0.40	2.43
S1-2	86.73	25.00	0.30	1.99
S2-1	86.73	25.00	0.28	1.76
S2-2	86.73	25.00	0.27	1.77
S3-1	86.73	25.00	0.28	1.79
S3-2	86.73	25.00	0.20	1.34
S4-1	86.73	25.00	0.35	2.25
S4-2	86.73	25.00	0.36	2.25

No.4 SRS	Known c (mg/l)	Measured c (mg/l)	Standard correction
Arabinose	100.40	92.96	0.93
Rhamnose	106.61	89.85	0.84
Galactose	204.00	186.80	0.92
Glucose	1025.00	943.29	0.92
Xylose	514.20	437.18	0.85
Mannose	99.30	78.65	0.79

No.5 Chemical components	Total sugars	Lignin	Extractive	Ash	Others
Salix Finland inner bark S1	0.37	0.19	0.18	0.07	0.19
Karin Sweden inner bark S2	0.39	0.19	0.18	0.05	0.19
Klara Sweden inner bark S3	0.35	0.19	0.21	0.04	0.20
Salix Russia inner bark S4	0.34	0.20	0.19	0.06	0.20

No.6-1 HPAEC- S1	measured c	measure 81 - a	measure 81 - b	correction ratio	C(anhydro)	sugar	OD sample (mg)
Arabinose	127.7762	125.6366	129.9158	0.88	126.07326	3.368 %	324.6194
Rhamnose	27.3843	24.7250	30.0435	0.9	26.18085	0.699 %	324.6194
Galactose	176.5711	176.4960	176.6462	0.9	183.15886	4.894 %	324.6194
Glucose	1189.4997	1187.3626	1191.6368	0.9	1147.3463	30.65 %	324.6194
Xylose	228.3555	226.3127	230.3982	0.88	239.36336	6.39 %	324.6194
Mannose	36.8195	36.9473	36.6917	0.9	38.420204	1.026 %	324.6194
					Total	47.03 %	

No.6-2 HPAEC – S2	measured c	measure 83 - a	measure 83 - b	correction ratio	C(anhydro)	sugar	OD sample (mg)
Arabinose	129.36	127.32	131.40	0.88	127.64	0.04	309.49
Rhamnose	34.91	34.45	35.38	0.90	33.38	0.01	309.49
Galactose	196.42	196.23	196.61	0.90	203.75	0.06	309.49
Glucose	1167.66	1163.31	1172.01	0.90	1126.28	0.32	309.49
Xylose	194.06	193.67	194.45	0.88	203.41	0.06	309.49
Mannose	50.90	51.91	49.89	0.90	53.11	0.01	309.49
					Total	48.98 %	

No.6-3 HPAEC – S3	measured c	measure 85 - a	measure 85 - b	correction ratio	C(anhydro)	sugar	OD sample (mg)
Arabinose	128.13	126.91	129.35	0.88	126.42	0.04	307.84
Rhamnose	30.57	30.41	30.73	0.90	29.23	0.01	307.84
Galactose	193.60	193.89	193.30	0.90	200.82	0.06	307.84
Glucose	1214.33	1215.82	1212.84	0.90	1171.29	0.33	307.84
Xylose	172.65	174.23	171.07	0.88	180.97	0.05	307.84
Mannose	51.96	53.13	50.79	0.90	54.21	0.02	307.84
					Total	49.67 %	

No.6-4 HPAEC – S4	measured c	measure 87 - a	measure 87 - b	correction ratio	C(anhydro)	sugar	OD sample (mg)
Arabinose	151.94	150.89	152.98	0.88	149.91	0.04	308.88
Rhamnose	35.82	35.23	36.42	0.90	34.25	0.01	308.88
Galactose	196.20	196.78	195.63	0.90	203.52	0.06	308.88
Glucose	1101.02	1109.16	1092.89	0.90	1062.00	0.30	308.88
Xylose	209.44	211.04	207.84	0.88	219.54	0.06	308.88
Mannose	40.90	39.52	42.28	0.90	42.68	0.01	308.88
					Total	48.07 %	

No.7 Specific sugar	Salix Finland inner bark S1	Karin Sweden inner bark S2	Klara Sweden inner bark S3	Salix Russia inner bark S4
Arabinose	0.033	0.036	0.037	0.041
Rhamnose	0.007	0.009	0.009	0.010
Galactose	0.048	0.058	0.057	0.056
Glucose	0.301	0.315	0.342	0.301
Xylose	0.063	0.057	0.054	0.063
Mannose	0.010	0.015	0.016	0.012

Code name: 20141210 WB Sample: four studied willow inner bark

S1-Salix Finland inner bark; S2- Karin Sweden inner bark; S3- Klara Sweden inner bark; S4 - Salix Russia inner bark

No.1 Extractive content	Aluminium (g)	Aluminium + dry sam (g)	Extractive (g)	O.D. original mass (g)	Extractive content
S1	1.12	1.92	0.80	4.44	0.18
S2	1.12	1.88	0.77	4.29	0.18
S3	1.12	1.64	0.53	2.46	0.21
S4	1.12	2.92	1.80	9.28	0.19

No.2 Klason lignin	Amount (mg)	OD (mg)	Crucible (g)	Crucible + Lignin (g)	Klason lignin (mg)	Klason (%)
S1-1	304.00	283.12	29.74	29.79	53.80	19.00
S1-2	307.30	282.32	29.68	29.74	58.50	20.72
S2-1	312.30	274.45	29.71	29.77	54.40	19.82
S2-2	316.30	288.53	29.69	29.75	58.30	20.21
S3-1	318.90	284.43	29.73	29.79	65.10	22.89
S3-2	312.00	291.61	29.71	29.77	61.10	20.95
S4-1	304.90	276.45	29.72	29.78	59.10	21.38
S4-2	305.70	278.16	29.65	29.71	61.00	21.93

No.3 ASL	V. (ml)	Dilution	Absorbance. 205 nm	ASL (%)
S1-1	86.73	25.00	0.48	3.34
S1-2	86.73	25.00	0.54	3.75
S2-1	86.73	25.00	0.42	3.02
S2-2	86.73	25.00	0.41	2.82
S3-1	86.73	25.00	0.39	2.70
S3-2	86.73	25.00	0.35	2.39
S4-1	86.73	25.00	0.49	3.47
S4-2	86.73	25.00	0.47	3.32

No.4 SRS	Known c (mg/l)	Measured c (mg/l)	Standard correction
Arabinose	100.40	92.96	0.93
Rhamnose	106.61	89.85	0.84
Galactose	204.00	186.80	0.92
Glucose	1025.00	943.29	0.92
Xylose	514.20	437.18	0.85
Mannose	99.30	78.65	0.79

No.5 Chemical components	Total sugars	Lignin	Extractive	Ash	Others
Salix Finland bark S1	0.37	0.19	0.18	0.07	0.19
Karin Sweden bark S2	0.39	0.19	0.18	0.05	0.19
Klara Sweden bark S3	0.35	0.19	0.21	0.04	0.20
Salix Russia bark S4	0.34	0.20	0.19	0.06	0.20

No.6-1 HPAEC- S1	measured c	measure 67 - a	measure 67 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	109.02	109.77	108.27	0.88	103.62	0.03	283.12
Rhamnose	18.21	18.44	17.97	0.90	19.44	0.01	283.12
Galactose	147.60	147.54	147.65	0.90	145.07	0.04	283.12
Glucose	1038.77	1040.57	1036.96	0.90	1015.87	0.31	283.12
Xylose	190.67	192.29	189.04	0.88	197.35	0.06	283.12
Mannose	28.24	28.68	27.79	0.90	32.09	0.01	283.12
					Total	46.36 %	

No.6-2 HPAEC – S2	measured c	measure 69 - a	measure 69 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	119.98	119.74	120.22	0.88	114.03	0.04	274.45
Rhamnose	22.20	22.37	22.04	0.90	23.71	0.01	274.45
Galactose	182.39	180.92	183.85	0.90	179.27	0.06	274.45
Glucose	1010.92	1007.26	1014.58	0.90	988.64	0.31	274.45
Xylose	146.21	145.60	146.82	0.88	151.33	0.05	274.45
Mannose	39.81	40.77	38.85	0.90	45.24	0.01	274.45
					Total	47.47 %	

No.6-3 HPAEC – S3	measured c	measure 71 - a	measure 71 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	114.33	114.18	114.48	0.88	108.66	0.03	284.43
Rhamnose	20.71	21.61	19.81	0.90	22.12	0.01	284.43
Galactose	175.06	177.14	172.97	0.90	172.07	0.05	284.43
Glucose	992.73	994.84	990.62	0.90	970.85	0.30	284.43
Xylose	113.99	115.35	112.64	0.88	117.99	0.04	284.43
Mannose	38.39	39.54	37.23	0.90	43.62	0.01	284.43
					Total	43.77 %	

No.6-4 HPAEC – S4	measured c	measure 73 - a	measure 73 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	110.54	108.83	112.25	0.88	105.06	0.03	276.45
Rhamnose	19.48	18.71	20.26	0.90	20.81	0.01	276.45
Galactose	150.07	151.70	148.44	0.90	147.51	0.05	276.45
Glucose	907.13	918.47	895.79	0.90	887.14	0.28	276.45
Xylose	156.35	159.70	153.01	0.88	161.83	0.05	276.45
Mannose	24.82	25.16	24.48	0.90	28.20	0.01	276.45
					Total	42.37 %	

No.7 Specific sugar	Salix Finland bark S1	Karin Sweden bark S2	Klara Sweden bark S3	Salix Russia bark S4
Arabinose	3.36	3.46	3.14	3.36
Rhamnose	0.59	0.75	0.63	0.66
Galactose	4.64	5.56	5.09	4.65
Glucose	29.66	31.28	30.51	27.66
Xylose	5.40	4.82	3.86	4.99
Mannose	0.98	1.44	1.34	0.87

Code name: 20141212 WB Sample: four studied willow inner bark

S1-Salix Finland inner bark; S2- Karin Sweden inner bark; S3- Klara Sweden inner bark; S4 - Salix Russia inner bark

No.1 Extractive content	Aluminium (g)	Aluminium + dry sam (g)	Extractive (g)	O.D. original mass (g)	Extractive content
S1	1.8735	3.1816	1.3081	6.00417943	0.217864908
S2	1.8733	3.3869	1.5136	6.428152458	0.235464235
S3	1.8787	3.5032	1.6245	6.613791756	0.245623095
S4	1.8809	3.3465	1.4656	6.431275262	0.227886374

No.2 Klason lignin	Amount (mg)	OD (mg)	Crucible (g)	Crucible + Lignin (g)	Klason lignin (mg)	Klason (%)
S1-1	304.00	280.84	29.70	29.76	54.00	19.23
S1-2	307.30	285.55	29.72	29.78	51.00	17.86
S2-1	312.30	287.38	29.71	29.76	47.80	16.63
S2-2	316.30	286.93	29.68	29.72	47.30	16.48
S3-1	318.90	294.08	29.65	29.70	53.30	18.12
S3-2	312.00	290.46	29.69	29.74	53.10	18.28
S4-1	304.90	282.24	29.72	29.76	48.80	17.29
S4-2	305.70	283.92	29.74	29.79	49.50	17.43

No.3 ASL	V. (ml)	Dilution	Absorbance. 205 nm	ASL (%)
S1-1	86.73	25.00	0.50	3.50
S1-2	86.73	25.00	0.55	3.82
S2-1	86.73	25.00	0.42	2.91
S2-2	86.73	25.00	0.43	2.94
S3-1	86.73	25.00	0.40	2.69
S3-2	86.73	25.00	0.40	2.74
S4-1	86.73	25.00	0.50	3.47
S4-2	86.73	25.00	0.52	3.62

No.4 SRS	Known c (mg/l)	Measured c (mg/l)	Standard correction
Arabinose	98.23	92.46	0.94
Rhamnose	108.64	102.43	0.94
Galactose	198.17	177.94	0.90
Glucose	1011.73	942.16	0.93
Xylose	498.52	415.80	0.83
Mannose	100.47	91.94	0.92

No.5 Chemical components	Total sugars	Lignin	Extractive	Ash	Others
Salix Finland bark S1	0.416	0.174	0.218	0.073	0.119
Karin Sweden bark S2	0.414	0.149	0.235	0.054	0.147
Klara Sweden bark S3	0.383	0.158	0.246	0.039	0.174
Salix Russia bark S4	0.353	0.161	0.228	0.061	0.197

No.6-1 HPAEC- S1	measured c	measure 89 - a	measure 89 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	95.96	95.96	95.96	0.88	89.71	0.03	280.84
Rhamnose	20.95	20.95	20.95	0.90	19.99	0.01	280.84
Galactose	106.22	106.22	106.22	0.90	106.47	0.03	280.84
Glucose	1187.40	1187.40	1187.40	0.90	1147.57	0.35	280.84
Xylose	211.87	211.87	211.87	0.88	223.54	0.07	280.84
Mannose	26.18	26.18	26.18	0.90	25.75	0.01	280.84
					Total	49.82 %	

No.6-2 HPAEC- S2	measured c	measure 91 - a	measure 91 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	124.93	124.93	124.93	0.88	116.80	0.04	287.38
Rhamnose	28.87	28.87	28.87	0.90	27.56	0.01	287.38
Galactose	123.84	123.84	123.84	0.90	124.12	0.04	287.38
Glucose	1309.77	1309.77	1309.77	0.90	1265.83	0.38	287.38
Xylose	201.65	201.65	201.65	0.88	212.76	0.06	287.38
Mannose	46.77	46.77	46.77	0.90	46.00	0.01	287.38
					Total	54.12 %	

No.6-3 HPAEC – S3	measured c	measure 93 - a	measure 93 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	129.10	129.10	129.10	0.88	120.70	0.04	294.08
Rhamnose	25.35	25.35	25.35	0.90	24.20	0.01	294.08
Galactose	148.39	148.39	148.39	0.90	148.74	0.04	294.08
Glucose	1271.42	1271.42	1271.42	0.90	1228.77	0.36	294.08
Xylose	151.73	151.73	151.73	0.88	160.08	0.05	294.08
Mannose	45.11	45.11	45.11	0.90	44.36	0.01	294.08
					Total	50.93 %	

No.6-4 HPAEC – S4	measured c	measure 95 - a	measure 95 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	114.79	114.79	114.79	0.88	107.32	0.03	282.24
Rhamnose	26.70	26.70	26.70	0.90	25.49	0.01	282.24
Galactose	112.07	112.07	112.07	0.90	112.33	0.03	282.24
Glucose	1032.54	1032.54	1032.54	0.90	997.90	0.31	282.24
Xylose	190.91	190.91	190.91	0.88	201.42	0.06	282.24
Mannose	21.86	21.86	21.86	0.90	21.50	0.01	282.24
					Total	45.05 %	

No.7 Specific sugar	Salix Finland inner bark S1	Karin Sweden inner bark S2	Klara Sweden inner bark S3	Salix Russia inner bark S4
Arabinose	2.83	3.54	3.61	3.47
Rhamnose	0.66	0.80	0.74	0.80
Galactose	3.41	3.68	4.35	3.43
Glucose	38.01	38.28	36.11	31.07
Xylose	7.48	6.46	4.61	6.25
Mannose	0.85	1.40	1.31	0.73

Code name: 20150107 WB Sample: four studied willow inner bark

S1-Salix Finland inner bark; S2- Karin Sweden inner bark; S3- Klara Sweden inner bark; S4 - Salix Russia inner bark

No.1 Extractive content	Aluminium (g)	Aluminium + dry sam (g)	Extractive (g)	O.D. original mass (g)	Extractive content
S1	1.87	2.59	0.72	4.69	0.15
S2	1.87	3.00	1.13	4.59	0.25
S3	1.90	2.94	1.04	4.52	0.23
S4	1.85	2.90	1.05	4.51	0.23

No.2 Klason lignin	Amount (mg)	OD (mg)	Crucible (g)	Crucible + Lignin (g)	Klason lignin (mg)	Klason %
S1-1	330.300	309.424	29.707	29.763	55.800	18.033
S1-2	336.700	314.550	29.723	29.785	61.200	19.456
S2-1	328.400	304.115	29.706	29.767	61.100	20.091
S2-2	322.700	300.446	29.679	29.734	55.300	18.406
S3-1	335.300	309.660	29.648	29.713	65.600	21.185
S3-2	339.200	316.525	29.685	29.752	66.700	21.073
S4-1	338.300	311.245	29.715	29.776	61.900	19.888
S4-2	332.900	311.990	29.734	29.794	60.900	19.520

No.3 ASL	V. (ml)	Dilution	Absorbance. 205 nm	ASL(%)
S1-1	86.730	25.000	0.449	2.860
S1-2	86.730	25.000	0.435	2.726
S2-1	86.730	25.000	0.411	2.664
S2-2	86.730	25.000	0.374	2.450
S3-1	86.730	25.000	0.322	2.047
S3-2	86.730	25.000	0.314	1.952
S4-1	86.730	25.000	0.364	2.302
S4-2	86.730	25.000	0.394	2.486

No.4 SRS	Known c (mg/l)	Measured c (mg/l)	Standard correction
Arabinose	98.230	92.458	0.941
Rhamnose	108.640	102.429	0.943
Galactose	198.170	177.941	0.898
Glucose	1011.730	942.161	0.931
Xylose	498.520	415.799	0.834
Mannose	100.470	91.941	0.915

No.5 Chemical components	Total sugars	Lignin	Extractive	Ash	Others
Salix Finland bark S1	0.447	0.182	0.155	0.073	0.143
Karin Sweden bark S2	0.392	0.164	0.247	0.054	0.143
Klara Sweden bark S3	0.426	0.178	0.230	0.039	0.127
Salix Russia bark S4	0.338	0.170	0.233	0.061	0.199

No.6-1 HPAEC- S1	measured c	measure 75 - a	measure 75 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	137.48	137.48	137.48	0.88	128.54	0.04	309.42
Rhamnose	27.84	27.84	27.84	0.90	26.57	0.01	309.42
Galactose	143.94	143.94	143.94	0.90	144.28	0.04	309.42
Glucose	1283.97	1283.97	1283.97	0.90	1240.90	0.35	309.42
Xylose	269.76	269.76	269.76	0.88	284.61	0.08	309.42
Mannose	43.94	43.94	43.94	0.90	43.21	0.01	309.42
					Total	52.36 %	

No.6-2 HPAEC –S2	measured c	measure 77 - a	measure 77 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	164.33	164.33	164.33	0.88	153.64	0.04	304.11
Rhamnose	35.71	35.71	35.71	0.90	34.09	0.01	304.11
Galactose	175.99	175.99	175.99	0.90	176.40	0.05	304.11
Glucose	1220.54	1220.54	1220.54	0.90	1179.60	0.34	304.11
Xylose	207.88	207.88	207.88	0.88	219.32	0.06	304.11
Mannose	59.79	59.79	59.79	0.90	58.81	0.02	304.11
					Total	51.96 %	

No.6-3 HPAEC – S3	measured c	measure 79 - a	measure 79 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	150.19	150.19	150.19	0.88	140.42	0.04	309.66
Rhamnose	29.62	29.62	29.62	0.90	28.27	0.01	309.66
Galactose	178.79	178.79	178.79	0.90	179.20	0.05	309.66
Glucose	1430.91	1430.91	1430.91	0.90	1382.91	0.39	309.66
Xylose	220.67	220.67	220.67	0.88	232.82	0.07	309.66
Mannose	60.31	60.31	60.31	0.90	59.31	0.02	309.66
					Total	56.66 %	

No.6-4 HPAEC – S4	measured c	measure 81 - a	measure 81 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	167.20	167.20	167.20	0.88	156.32	0.04	311.24
Rhamnose	35.66	35.66	35.66	0.90	34.04	0.01	311.24
Galactose	168.18	168.18	168.18	0.90	168.57	0.05	311.24
Glucose	1214.73	1214.73	1214.73	0.90	1173.98	0.33	311.24
Xylose	257.01	257.01	257.01	0.88	271.17	0.08	311.24
Mannose	39.33	39.33	39.33	0.90	38.68	0.01	311.24
					Total	51.35 %	

No.7 Specific sugar	Salix Finland inner bark S1	Karin Sweden inner bark S2	Klara Sweden inner bark S3	Salix Russia inner bark S4
Arabinose	3.66	4.37	4.04	3.69
Rhamnose	0.76	0.96	0.83	0.80
Galactose	4.12	5.01	5.09	3.98
Glucose	35.19	33.83	37.55	28.12
Xylose	7.98	6.24	6.18	6.53
Mannose	1.21	1.64	1.61	0.89

Code name: 20150108 WB Sample: four studied willow inner bark

S1-Salix Finland inner bark; S2- Karin Sweden inner bark; S3- Klara Sweden inner bark; S4 - Salix Russia inner bark

No.1 Extractive content	Aluminium (g)	Aluminium + dry sam (g)	Extractive (g)	O.D. original mass (g)	Extractive content
S1	1.87	2.95	1.09	4.79	0.23
S2	1.88	2.88	1.00	4.68	0.21
S3	1.89	3.03	1.14	4.89	0.23
S4	1.97	2.66	0.70	3.07	0.23

No.2 Klason lignin	Amount (mg)	OD (mg)	Crucible (g)	Crucible + Lignin (g)	Klason lignin (mg)	Klason (%)
S1-1	327.40	300.89	29.77	29.83	58.10	19.31
S1-2	345.90	314.74	29.57	29.63	61.00	19.38
S2-1	336.30	309.63	29.54	29.61	64.80	20.93
S2-2	346.60	319.03	29.63	29.70	64.10	20.09
S3-1	360.40	334.35	29.29	29.37	72.70	21.74
S3-2	352.40	327.57	29.40	29.47	71.80	21.92
S4-1	341.90	313.46	29.20	29.27	63.50	20.26
S4-2	337.30	291.50	29.09	29.15	60.00	20.58

No.3 ASL	V. (ml)	Dilution	Absorbance. 205 nm	ASL (%)
S1-1	86.73	25.00	0.48	3.16
S1-2	86.73	25.00	0.44	2.75
S2-1	86.73	25.00	0.45	2.87
S2-2	86.73	25.00	0.44	2.73
S3-1	86.73	25.00	0.51	2.99
S3-2	86.73	25.00	0.36	2.19
S4-1	86.73	25.00	0.51	3.18
S4-2	86.73	25.00	0.48	3.27

No.4 SRS	Known c (mg/l)	Measured c (mg/l)	Standard correction
Arabinose	98.23	92.46	0.94
Rhamnose	108.64	102.43	0.94
Galactose	198.17	177.94	0.90
Glucose	1011.73	942.16	0.93
Xylose	498.52	415.80	0.83
Mannose	100.47	91.94	0.92

No.5 Chemical components	Total sugars	Lignin	Extractive	Ash	Others
Salix Finland bark S1	0.402	0.172	0.227	0.073	0.126
Karin Sweden bark S2	0.389	0.183	0.213	0.054	0.160
Klara Sweden bark S3	0.421	0.187	0.233	0.039	0.119
Salix Russia bark S4	0.353	0.183	0.227	0.061	0.176

No.6-1 HPAEC- S1	measured c	measure 85 - a	measure 85 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	140.31	140.31	140.31	0.88	131.19	0.04	300.89
Rhamnose	28.59	28.59	28.59	0.90	27.29	0.01	300.89
Galactose	149.41	149.41	149.41	0.90	149.75	0.04	300.89
Glucose	1267.35	1267.35	1267.35	0.90	1224.83	0.35	300.89
Xylose	262.48	262.48	262.48	0.88	276.94	0.08	300.89
Mannose	43.69	43.69	43.69	0.90	42.97	0.01	300.89
					Total	53.41 %	

No.6-2 HPAEC – S2	measured c	measure 87 - a	measure 87 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	134.50	134.50	134.50	0.88	125.75	0.04	309.63
Rhamnose	32.11	32.11	32.11	0.90	30.65	0.01	309.63
Galactose	168.83	168.83	168.83	0.90	169.22	0.05	309.63
Glucose	1215.35	1215.35	1215.35	0.90	1174.59	0.33	309.63
Xylose	197.72	197.72	197.72	0.88	208.61	0.06	309.63
Mannose	51.90	51.90	51.90	0.90	51.05	0.01	309.63
					Total	49 %	

No.6-3 HPAEC – S3	measured c	measure 89 - a	measure 89 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	164.26	164.26	164.26	0.88	153.58	0.04	334.35
Rhamnose	39.35	39.35	39.35	0.90	37.57	0.01	334.35
Galactose	218.85	218.85	218.85	0.90	219.36	0.06	334.35
Glucose	1715.34	1715.34	1715.34	0.90	1657.80	0.43	334.35
Xylose	240.58	240.58	240.58	0.88	253.83	0.07	334.35
Mannose	74.02	74.02	74.02	0.90	72.80	0.02	334.35
					Total	62.12 %	

No.6-4 HPAEC – S4	measured c	measure 91 - a	measure 91 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	135.42	135.42	135.42	0.88	126.60	0.04	313.46
Rhamnose	33.03	33.03	33.03	0.90	31.53	0.01	313.46
Galactose	152.23	152.23	152.23	0.90	152.58	0.04	313.46
Glucose	1083.14	1083.14	1083.14	0.90	1046.81	0.29	313.46
Xylose	219.77	219.77	219.77	0.88	231.87	0.06	313.46
Mannose	36.57	36.57	36.57	0.90	35.96	0.01	313.46
					Total	44.97 %	

No.7 Specific sugar	Salix Finland inner bark S1	Karin Sweden inner bark S2	Klara Sweden inner bark S3	Salix Russia inner bark S4
Arabinose	3.64	3.58	3.55	3.51
Rhamnose	0.76	0.87	0.87	0.85
Galactose	4.19	4.77	5.05	4.20
Glucose	34.47	32.86	38.01	29.49
Xylose	7.69	5.95	5.76	6.67
Mannose	1.20	1.41	1.67	0.97

Code name: 20150113 WB Sample: four studied willow inner bark

S1-Salix Finland inner bark; S2- Karin Sweden inner bark; S3- Klara Sweden inner bark; S4 - Salix Russia inner bark

No.1 Extractive content	Aluminium (g)	Aluminium + dry sam (g)	Extractive (g)	O.D. original mass (g)	Extractive content
S1	1.86	2.90	2.31	10.65	0.22
S2	1.85	3.12	2.43	9.78	0.25
S3	1.86	3.18	1.58	7.41	0.21
S4	1.86	2.61	0.99	6.59	0.15

No.2 Klason lignin	Amount (mg)	OD (mg)	Crucible (g)	Crucible + Lignin (g)	Klason lignin (mg)	Klason (%)
S1-1	315.90	296.28	19.80	19.85	53.90	18.19
S1-2	326.37	306.47	24.50	24.55	58.60	19.12
S2-1	334.36	312.37	29.21	29.27	63.60	20.36
S2-2	321.60	299.91	29.54	29.61	66.40	22.14
S3-1	339.57	323.37	19.99	20.06	66.30	20.50
S3-2	346.60	330.07	23.48	23.55	69.40	21.03
S4-1	349.70	330.66	29.63	29.70	70.90	21.44
S4-2	337.28	318.92	29.31	29.37	66.30	20.79

No.3 ASL	V.(ml)	Dilution	Absorbance. 205 nm	ASL (%)
S1-1	86.73	25.00	0.46	3.09
S1-2	86.73	25.00	0.45	2.87
S2-1	86.73	25.00	0.36	2.25
S2-2	86.73	25.00	0.34	2.21
S3-1	86.73	25.00	0.41	2.49
S3-2	86.73	25.00	0.41	2.44
S4-1	86.73	25.00	0.50	2.96
S4-2	86.73	25.00	0.48	2.94

No.4 SRS	Known c (mg/l)	Measured c (mg/l)	Standard correction
Arabinose	98.230	92.458	0.941
Rhamnose	108.640	102.429	0.943
Galactose	198.170	177.941	0.898
Glucose	1011.730	942.161	0.931
Xylose	498.520	415.799	0.834
Mannose	100.470	91.941	0.915

No.5 Chemical components	Total sugars	Lignin	Extractive	Ash	Others
Salix Finland bark S1	0.41	0.17	0.22	0.07	0.13
Karin Sweden bark S2	0.40	0.18	0.25	0.05	0.12
Klara Sweden bark S3	0.38	0.18	0.21	0.04	0.18
Salix Russia bark S4	0.40	0.20	0.15	0.06	0.19

No.6-1 HPAEC- S1	measured c	measure 40 - a	measure 40 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	117.45	111.33	123.56	0.88	109.80	0.03	296.46
Rhamnose	25.73	25.20	26.26	0.90	24.56	0.01	296.46
Galactose	146.51	140.43	152.59	0.90	146.85	0.04	296.46
Glucose	1241.03	1216.01	1266.05	0.90	1199.40	0.35	296.46
Xylose	297.57	277.68	317.47	0.88	313.96	0.09	296.46
Mannose	38.29	33.26	43.33	0.90	37.66	0.01	296.46
					total	53.61 %	

No.6-2 HPAEC–S2	measured c	measure 42 - a	measure 42 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	133.60	133.02	134.19	0.88	124.91	0.03	312.37
Rhamnose	30.67	30.80	30.54	0.90	29.28	0.01	312.37
Galactose	185.70	185.11	186.30	0.90	186.13	0.05	312.37
Glucose	1326.63	1324.31	1328.94	0.90	1282.13	0.36	312.37
Xylose	226.64	226.10	227.19	0.88	239.13	0.07	312.37
Mannose	57.14	57.68	56.59	0.90	56.19	0.02	312.37
					total	53.25 %	

No.6-3 HPAEC – S3	measured c	measure 44 - a	measure 44 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	136.14	135.73	136.56	0.88	127.29	0.03	323.37
Rhamnose	31.17	32.27	30.07	0.90	29.76	0.01	323.37
Galactose	191.58	191.38	191.77	0.90	192.02	0.05	323.37
Glucose	1216.18	1211.71	1220.65	0.90	1175.38	0.32	323.37
Xylose	221.82	218.35	225.28	0.88	234.03	0.06	323.37
Mannose	52.91	52.23	53.58	0.90	52.03	0.01	323.37
					total	48.56 %	

No.6-4 HPAEC – S4	measured c	measure 46 - a	measure 46 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	154.37	154.72	154.01	0.88	144.32	0.04	330.66
Rhamnose	35.77	35.38	36.15	0.90	34.14	0.01	330.66
Galactose	223.72	223.43	224.01	0.90	224.24	0.06	330.66
Glucose	1174.85	1173.65	1176.06	0.90	1135.44	0.30	330.66
Xylose	233.99	232.78	235.20	0.88	246.88	0.06	330.66
Mannose	39.48	39.91	39.06	0.90	38.83	0.01	330.66
					total	48 %	

No.7 Specific sugar	Salix Finland inner bark S1	Karin Sweden inner bark S2	Klara Sweden inner bark S3	Salix Russia inner bark S4
Arabinose	3.01	3.48	3.50	3.68
Rhamnose	0.70	0.82	0.83	0.87
Galactose	4.10	5.17	5.24	5.76
Glucose	34.90	35.61	31.47	29.30
Xylose	9.01	6.65	6.17	6.39
Mannose	1.05	1.59	1.36	0.99

Code name: 20150108 WW Sample: four studied willow wood

S1-Salix Finland wood; S2- Karin Sweden wood; S3- Klara Sweden wood; S4 - Salix Russia wood

No.1 Extractive content	Aluminium (g)	Aluminium + dry sam (g)	Extractive (g)	O.D. original mass (g)	Extractive content
S1	1.88	2.20	0.32	9.73	0.03
S2	1.87	2.11	0.24	9.81	0.02
S3	1.88	2.18	0.30	9.87	0.03
S4	1.87	2.07	0.19	9.83	0.02

No.2 Klason lignin	Amount (mg)	OD (mg)	Crucible (g)	Crucible + Lignin (g)	Klason lignin (mg)	Klason (%)
S1-1	339.40	325.40	29.71	29.79	78.10	24.00
S1-2	327.80	316.00	29.73	29.80	73.20	23.16
S2-1	339.70	321.09	29.71	29.79	74.50	23.20
S2-2	335.80	319.35	29.68	29.75	72.50	22.70
S3-1	340.20	321.92	29.65	29.72	72.70	22.58
S3-2	333.20	320.75	29.69	29.79	96.10	29.96
S4-1	341.00	327.87	29.72	29.76	39.60	12.08
S4-2	336.30	323.42	29.73	29.80	69.60	21.52

No.3 ASL	V. (ml)	Dilution	Absorbance. 205 nm	ASL (%)
S1-1	86.730	25.000	0.401	2.426
S1-2	86.730	25.000	0.394	2.458
S2-1	86.730	25.000	0.459	2.815
S2-2	86.730	25.000	0.416	2.568
S3-1	86.730	25.000	0.438	2.679
S3-2	86.730	25.000	0.407	2.501
S4-1	86.730	25.000	0.397	2.387
S4-2	86.730	25.000	0.372	2.267

No.4 SRS	Known c (mg/l)	Measured c (mg/l)	Standard correction
Arabinose	98.23	92.46	0.94
Rhamnose	108.64	102.43	0.94
Galactose	198.17	177.94	0.90
Glucose	1011.73	942.16	0.93
Xylose	498.52	415.80	0.83
Mannose	100.47	91.94	0.92

No.5 Chemical components	Total sugars	Lignin	Extractive	Ash	Others
Salix Finland wood S1	0.561	0.252	0.033	0.006	0.149
Karin Sweden wood S2	0.559	0.250	0.024	0.005	0.161
Klara Sweden wood S3	0.585	0.280	0.030	0.005	0.100
Salix Russia wood S4	0.565	0.187	0.020	0.005	0.223

No.6-1 HPAEC-S1	measured c	measure 97 - a	measure 97 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	18.444	17.813	19.076	0.880	17.244	0.005	325.399
Rhamnose	15.169	15.896	14.443	0.900	14.480	0.004	325.399
Galactose	41.081	41.094	41.068	0.900	41.176	0.011	325.399
Glucose	1513.025	1514.592	1511.458	0.900	1462.271	0.390	325.399
Xylose	560.442	560.778	560.107	0.880	591.307	0.158	325.399
Mannose	64.019	65.306	62.732	0.900	62.962	0.017	325.399
					total	58.35 %	

No.6-2 HPAEC -S2	measured c	measure 99 - a	measure 99 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	18.609	18.358	18.861	0.880	17.398	0.005	321.089
Rhamnose	17.391	16.949	17.832	0.900	16.600	0.004	321.089
Galactose	37.132	36.985	37.279	0.900	37.218	0.010	321.089
Glucose	1455.568	1455.132	1456.005	0.900	1406.742	0.380	321.089
Xylose	583.262	584.657	581.867	0.880	615.384	0.166	321.089
Mannose	78.151	76.951	79.351	0.900	76.861	0.021	321.089
					total	58.6%	

No.6-3 HPAEC – S3	measured c	measure 101 - a	measure 101 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	15.83	16.30	15.35	0.88	14.80	0.00	321.92
Rhamnose	16.00	16.60	15.41	0.90	15.28	0.00	321.92
Galactose	36.04	35.41	36.67	0.90	36.13	0.01	321.92
Glucose	1565.81	1564.05	1567.57	0.90	1513.29	0.41	321.92
Xylose	553.93	555.31	552.55	0.88	584.44	0.16	321.92
Mannose	64.07	63.24	64.90	0.90	63.01	0.02	321.92
					total	60%	

No.6-4 HPAEC – S4	measured c	measure 103 - a	measure 103 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	15.324	15.792	14.856	0.880	14.327	0.004	327.869
Rhamnose	15.122	15.261	14.983	0.900	14.435	0.004	327.869
Galactose	28.105	27.817	28.393	0.900	28.170	0.007	327.869
Glucose	1461.415	1460.894	1461.935	0.900	1412.393	0.374	327.869
Xylose	609.704	609.678	609.730	0.880	643.282	0.170	327.869
Mannose	51.087	51.271	50.902	0.900	50.243	0.013	327.869
					total	57.2%	

No.7 Specific sugar	Salix Finland wood S1	Karin Sweden wood S2	Klara Sweden wood S3	Salix Russia wood S4
Arabinose	0.46	0.45	0.40	0.38
Rhamnose	0.39	0.44	0.40	0.39
Galactose	1.07	0.99	0.98	0.75
Glucose	38.77	37.17	41.02	37.61
Xylose	15.67	16.27	15.79	17.14
Mannose	1.64	2.02	1.70	1.35

Code name: 20150109 WW Sample: four studied willow wood

S1-Salix Finland wood; S2- Karin Sweden wood; S3- Klara Sweden wood; S4 - Salix Russia wood

No.1 Extractive content	Aluminium (g)	Aluminium + dry sam (g)	Extractive (g)	O. D sample (g)	Extractive content
S1	1.86	2.02	0.16	5.20	0.03
S2	1.86	1.98	0.12	5.39	0.02
S3	1.88	2.05	0.17	5.38	0.03
S4	1.89	2.02	0.14	5.58	0.02

No.2 Klason lignin	Amount (mg)	OD (mg)	Crucible (g)	Crucible + Lignin (g)	Klason lignin (mg)	Klason (%)
S1-1	335.30	304.62	28.86	28.93	76.70	25.18
S1-2	328.20	304.32	29.68	29.75	65.10	21.39
S2-1	344.60	314.30	29.05	29.13	75.70	24.09
S2-2	330.00	304.02	28.65	28.72	69.80	22.96
S3-1	353.60	325.32	29.79	29.87	73.30	22.53
S3-2	348.00	323.07	29.74	29.81	71.70	22.19
S4-1	350.30	327.17	29.20	29.27	77.90	23.81
S4-2	347.80	317.56	29.30	29.37	72.30	22.77

No.3 ASL	V. (ml)	Dilution	Absorbance. 205 nm	ASL (%)
S1-1	86.73	25.00	0.38	2.47
S1-2	86.73	25.00	0.39	2.50
S2-1	86.73	25.00	0.42	2.64
S2-2	86.73	25.00	0.40	2.56
S3-1	86.73	25.00	0.40	2.43
S3-2	86.73	25.00	0.45	2.75
S4-1	86.73	25.00	0.38	2.30
S4-2	86.73	25.00	0.38	2.37

No.4 SRS	Known c (mg/l)	Measured c (mg/l)	Standard correction
Arabinose	98.23	92.46	0.94
Rhamnose	108.64	102.43	0.94
Galactose	198.17	177.94	0.90
Glucose	1011.73	942.16	0.93
Xylose	498.52	415.80	0.83
Mannose	100.47	91.94	0.92

No.5 Chemical components	Total sugars	Lignin	Extractive	Ash	Others
Salix Finland wood S1	0.599	0.250	0.031	0.006	0.115
Karin Sweden wood S2	0.601	0.255	0.023	0.005	0.116
Klara Sweden wood S3	0.607	0.242	0.031	0.005	0.115
Salix Russia wood S4	0.609	0.250	0.024	0.005	0.112

No.6-1 HPAEC-S1	measured c	measure 10 - a	measure 10 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	19.77	19.77	19.77	0.88	18.49	0.01	304.62
Rhamnose	16.10	16.10	16.10	0.90	15.37	0.00	304.62
Galactose	39.40	39.40	39.40	0.90	39.49	0.01	304.62
Glucose	1490.08	1490.08	1490.08	0.90	1440.09	0.41	304.62
Xylose	557.45	557.45	557.45	0.88	588.15	0.17	304.62
Mannose	68.19	68.19	68.19	0.90	67.07	0.02	304.62
					Total	61.74 %	

No.6-2 HPAEC -S2	measured c	measure 12 - a	measure 12 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	19.70	19.70	19.70	0.88	18.42	0.01	314.30
Rhamnose	19.45	19.45	19.45	0.90	18.57	0.01	314.30
Galactose	37.81	37.81	37.81	0.90	37.90	0.01	314.30
Glucose	1486.11	1486.11	1486.11	0.90	1436.26	0.40	314.30
Xylose	608.20	608.20	608.20	0.88	641.69	0.18	314.30
Mannose	85.87	85.87	85.87	0.90	84.45	0.02	314.30
					Total	62 %	

No.6-3 HPAEC- S3	measured c	measure 14 - a	measure 14 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	16.13	16.13	16.13	0.88	15.08	0.00	325.32
Rhamnose	18.33	18.33	18.33	0.90	17.50	0.00	325.32
Galactose	36.69	36.69	36.69	0.90	36.78	0.01	325.32
Glucose	1638.45	1638.45	1638.45	0.90	1583.49	0.42	325.32
Xylose	598.51	598.51	598.51	0.88	631.47	0.17	325.32
Mannose	70.53	70.53	70.53	0.90	69.37	0.02	325.32
					Total	63 %	

No.6-4 HPAEC–S4	measured c	measure 16 - a	measure 16 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	17.092	17.092	17.092	0.880	15.980	0.004	327.171
Rhamnose	16.726	16.726	16.726	0.900	15.966	0.004	327.171
Galactose	30.056	30.056	30.056	0.900	30.125	0.008	327.171
Glucose	1566.171	1566.171	1566.171	0.900	1513.635	0.401	327.171
Xylose	671.128	671.128	671.128	0.880	708.088	0.188	327.171
Mannose	61.847	61.847	61.847	0.900	60.826	0.016	327.171
					Total	62.2 %	

No.7 Specific sugar	Salix Finland wood S1	Karin Sweden wood S2	Klara Sweden wood S3	Salix Russia wood S4
Arabinose	0.53	0.50	0.40	0.44
Rhamnose	0.44	0.51	0.46	0.44
Galactose	1.13	1.05	0.99	0.79
Glucose	41.06	39.48	42.16	40.37
Xylose	16.73	17.64	16.78	18.83
Mannose	1.87	2.31	1.83	1.58

Code name: 20150114 WW Sample: four studied willow wood

S1-Salix Finland wood; S2- Karin Sweden wood; S3- Klara Sweden wood; S4 - Salix Russia wood

No.1 Extractive content	Aluminium (g)	Aluminium + dry sam (g)	Extractive(g)	O. D sample (g)	Extractive content
S1	1.84	2.18	0.34	9.74	0.03
S2	1.87	2.09	0.22	9.80	0.02
S3	1.87	2.22	0.35	10.70	0.03
S4	1.87	2.06	0.19	10.14	0.02

No.2 Klason lignin	Amount (mg)	OD (mg)	Crucibles	Crucible (g)	Crucible + Lignin (g)	Klason lignin (mg)	Klason (%)
S1-1	351.20	325.54	9.00	29.77	29.85	77.40	23.78
S1-2	328.90	305.55	10.00	29.27	29.34	75.90	24.84
S2-1	343.60	319.15	11.00	29.54	29.61	71.50	22.40
S2-2	347.60	320.01	12.00	29.63	29.71	76.80	24.00
S3-1	347.00	321.53	13.00	29.40	29.47	74.10	23.05
S3-2	328.40	304.42	14.00	29.57	29.64	70.90	23.29
S4-1	358.80	329.91	15.00	29.20	29.28	75.60	22.92
S4-2	347.80	320.76	16.00	29.09	29.16	72.00	22.45

Sample	V. (ml)	Dilution	Absorbance. 205 nm	ASL (%)
S1-1	86.73	25.00	0.40	2.45
S1-2	86.73	25.00	0.42	2.74
S2-1	86.73	25.00	0.48	2.96
S2-2	86.73	25.00	0.46	2.81
S3-1	86.73	25.00	0.48	2.94
S3-2	86.73	25.00	0.45	2.94
S4-1	86.73	25.00	0.43	2.55
S4-2	86.73	25.00	0.42	2.56

No.4 SRS	Known c (mg/l)	Measured c (mg/l)	Standard correction
Arabinose	98.23	92.46	0.94
Rhamnose	108.64	102.43	0.94
Galactose	198.17	177.94	0.90
Glucose	1011.73	942.16	0.93
Xylose	498.52	415.80	0.83
Mannose	100.47	91.94	0.92

No.5 Chemical components	Total sugars	Lignin	Extractive	Ash	Others
Salix Finland wood S1	0.65	0.26	0.03	0.01	0.05
Karin Sweden wood S2	0.65	0.26	0.02	0.01	0.07
Klara Sweden wood S3	0.63	0.25	0.03	0.01	0.08
Salix Russia wood S4	0.64	0.25	0.02	0.00	0.09

No.6-1 HPAEC-S1	measured c	measure 36 - a	measure 36 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	22.80	22.80	22.80	0.88	21.32	0.01	325.54
Rhamnose	18.78	18.78	18.78	0.90	17.92	0.00	325.54
Galactose	48.39	48.39	48.39	0.90	48.51	0.01	325.54
Glucose	1755.41	1755.41	1755.41	0.90	1696.53	0.45	325.54
Xylose	628.59	628.59	628.59	0.88	663.21	0.18	325.54
Mannose	78.21	78.21	78.21	0.90	76.92	0.02	325.54
					Total	67.26 %	

No.6-2 HPAEC -S2	measured c	measure 38 - a	measure 38- b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	21.22	21.22	21.22	0.88	19.84	0.01	319.15
Rhamnose	19.96	19.96	19.96	0.90	19.06	0.01	319.15
Galactose	40.93	40.93	40.93	0.90	41.03	0.01	319.15
Glucose	1611.66	1611.66	1611.66	0.90	1557.60	0.42	319.15
Xylose	645.46	645.46	645.46	0.88	681.01	0.19	319.15
Mannose	92.63	92.63	92.63	0.90	91.10	0.02	319.15
					Total	65.48 %	

No.6-3 HPAEC – S3	measured c	measure 40- a	measure 40 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	16.567	16.567	16.567	0.880	15.489	0.004	321.526
Rhamnose	17.685	17.685	17.685	0.900	16.882	0.005	321.526
Galactose	38.073	38.073	38.073	0.900	38.161	0.010	321.526
Glucose	1713.599	1713.599	1713.599	0.900	1656.118	0.447	321.526
Xylose	621.600	621.600	621.600	0.880	655.833	0.177	321.526
Mannose	74.376	74.376	74.376	0.900	73.148	0.020	321.526
					Total	66.2 %	

No.6-4 HPAEC – S4	measured c	measure 42 - a	measure 42 - b	correction ratio	C(anhydro)	Sugar	OD sample (mg)
Arabinose	19.414	19.414	19.414	0.880	18.151	0.005	329.908
Rhamnose	18.906	18.906	18.906	0.900	18.047	0.005	329.908
Galactose	30.625	30.625	30.625	0.900	30.696	0.008	329.908
Glucose	1675.191	1675.191	1675.191	0.900	1618.998	0.426	329.908
Xylose	704.110	704.110	704.110	0.880	742.886	0.195	329.908
Mannose	62.453	62.453	62.453	0.900	61.422	0.016	329.908
					Total	65.47 %	

No.7 Specific sugar	Salix Finland wood S1	Karin Sweden wood S2	Klara Sweden wood S3	Salix Russia wood S4
Arabinose	0.56	0.52	0.41	0.49
Rhamnose	0.50	0.52	0.45	0.49
Galactose	1.28	1.09	1.03	0.82
Glucose	45.02	42.06	44.39	42.74
Xylose	17.62	18.39	17.54	19.62
Mannose	2.00	2.43	2.00	1.66

4.3 Conclusion about the sugar composition

Table 4-3-1. Chemical components (A) and specific sugar components (B) of the inner bark section from the four willow clones- in % of total sugar (STD= standard deviation).

A	Salix Finland S1 (%)	STD S1	Karin Sweden S2 (%)	STD S2	Klara Sweden S3 (%)	STD S3	Salix Russia S4 (%)	STD S4
Total sugars	40.73	2.80	39.87	1.76	39.97	2.95	36.86	2.92
Lignin	18.01	0.84	17.09	1.33	18.11	1.13	18.45	1.57
Extractive	19.32	2.72	22.62	2.41	22.67	1.31	20.50	2.91
Ash	7.32	0.48	5.42	0.22	3.95	0.33	6.07	1.21
Others	14.62	2.86	15.01	2.66	15.30	3.69	18.12	2.26

B	Salix Finland S1 (%)	STD S1	Karin Sweden S2 (%)	STD S2	Klara Sweden S3 (%)	STD S3	Salix Russia S4 (%)	STD S4
Arabinose	6.60	0.31	7.33	0.42	7.09	0.35	8.09	0.38
Rhamnose	1.39	0.07	1.71	0.10	1.57	0.10	1.85	0.12
Galactose	8.42	0.46	9.81	0.70	9.91	0.42	10.08	0.86
Glucose	66.79	2.94	66.18	2.54	67.86	3.10	64.12	1.62
Xylose	14.60	1.18	11.85	0.72	10.46	0.87	13.70	0.70
Mannose	2.21	0.22	3.11	0.31	3.11	0.37	2.16	0.22

Table 4-3-2. Chemical components (A) and specific sugar components (B) of the wood section from the four willow clones- in % of total sugar (STD= standard deviation).

A	Salix Finland S1 (%)	STD S1	Karin Sweden S2 (%)	STD S2	Klara Sweden S3 (%)	STD S3	Salix Russia S4 (%)	STD S4
Total sugars	60.21	4.29	60.22	4.34	60.67	2.21	60.53	3.86
Lignin	25.37	0.53	25.35	0.29	25.80	1.97	22.84	3.54
Extractive	3.26	0.19	2.32	0.11	3.14	0.13	2.09	0.30
Ash	0.57	0.00	0.53	0.19	0.51	0.09	0.45	0.09
Others	10.58	4.84	11.59	4.47	9.87	1.73	14.09	7.25

B	Salix Finland S1 (%)	STD S1	Karin Sweden S2 (%)	STD S2	Klara Sweden S3 (%)	STD S3	Salix Russia S4 (%)	STD S4
Arabinose	0.83	0.05	0.80	0.04	0.64	0.00	0.71	0.05
Rhamnose	0.71	0.06	0.80	0.04	0.70	0.03	0.71	0.05
Galactose	1.86	0.11	1.70	0.05	1.59	0.03	1.27	0.03
Glucose	66.86	3.16	64.57	2.45	67.60	1.72	64.94	2.57
Xylose	26.79	0.98	28.45	1.07	26.55	0.88	29.90	1.27
Mannose	2.95	0.18	3.68	0.21	2.93	0.15	2.47	0.16

Table 4-3-3. Comparison between the wood and bark components from the four studied willow clones.

Sample	Sugar (%)		Lignin (%)		Extractive (%)		Ash (%)	
	Wood	Inner bark	Wood	Inner bark	Wood	Inner bark	Wood	Inner bark
Salix Finland S1	60.21	40.73	25.37	18.01	3.26	19.32	0.57	7.32
Karin Sweden S2	60.22	39.87	25.35	17.09	2.32	22.62	0.53	5.42
Klara Sweden S3	60.67	39.97	25.80	18.11	3.14	22.67	0.51	3.95
Salix Russia S4	60.53	36.86	22.84	18.45	2.09	20.50	0.45	6.07

5 IR spectroscopy

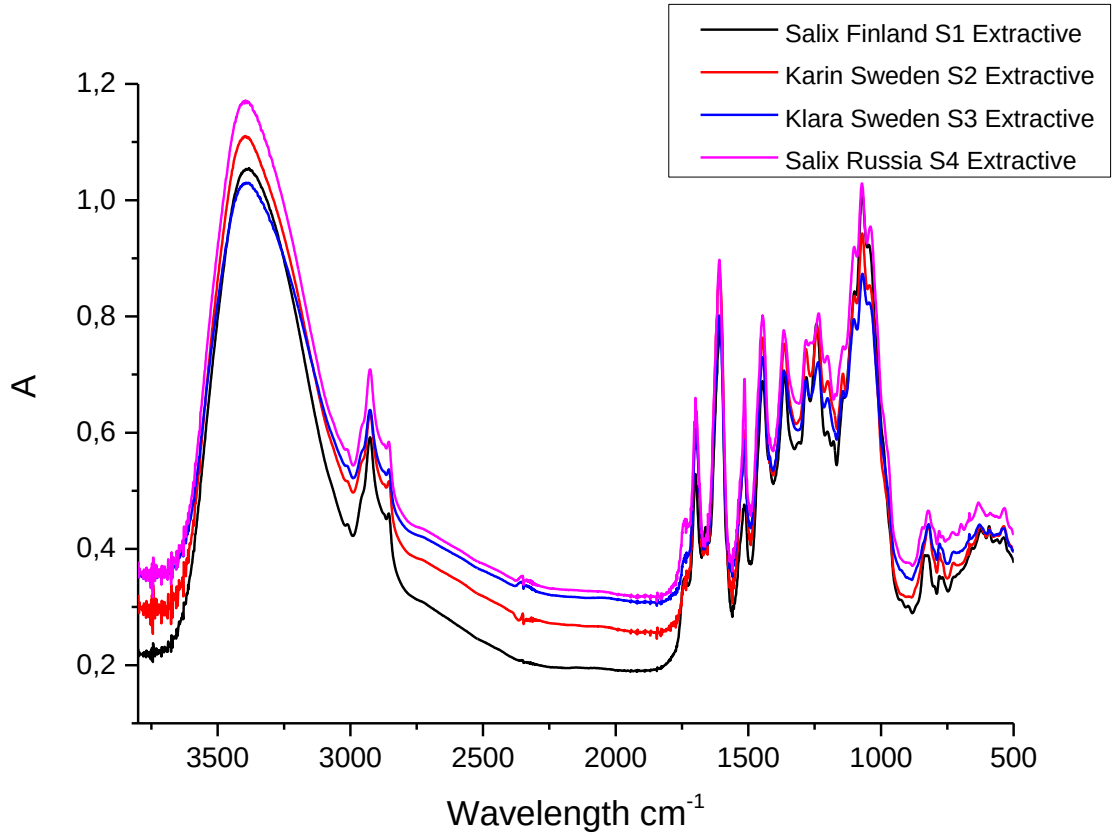

Figure 5-1. IR spectroscopy of four studied willow species.

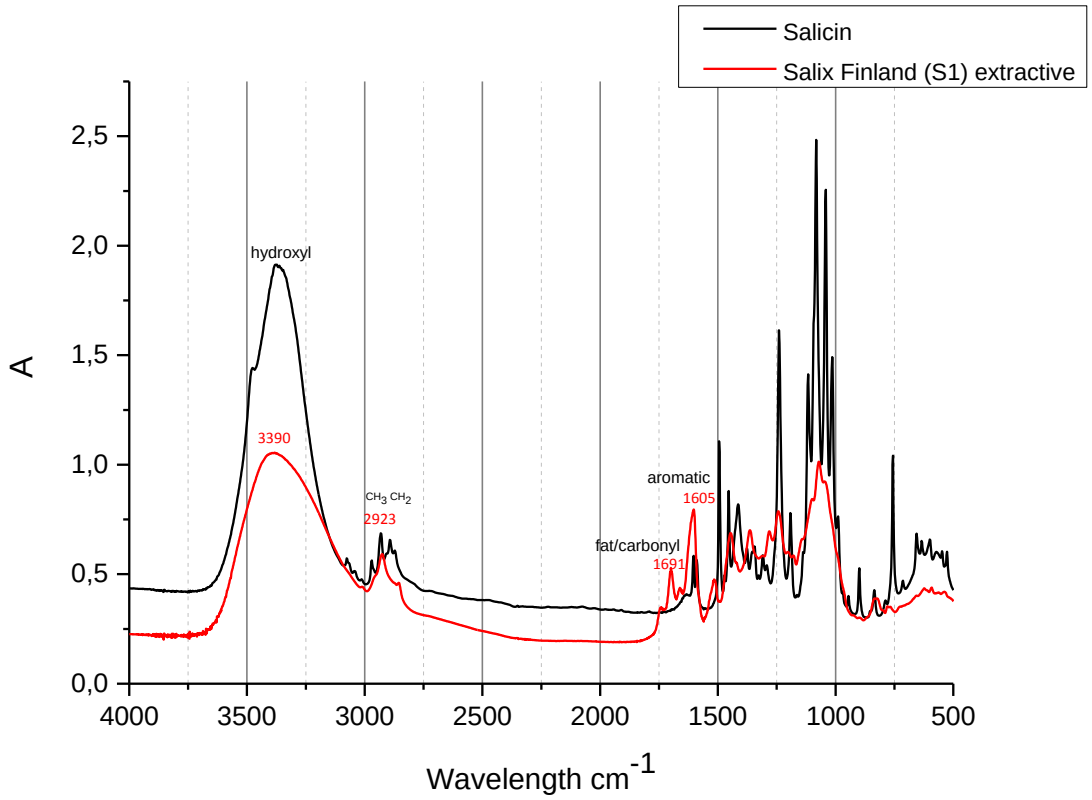

Figure 5-2. Infrared (IR) spectroscopy of the willow inner bark (S1 Finland) extractives and the salicin.

6 UV resonance Raman (UVRR)

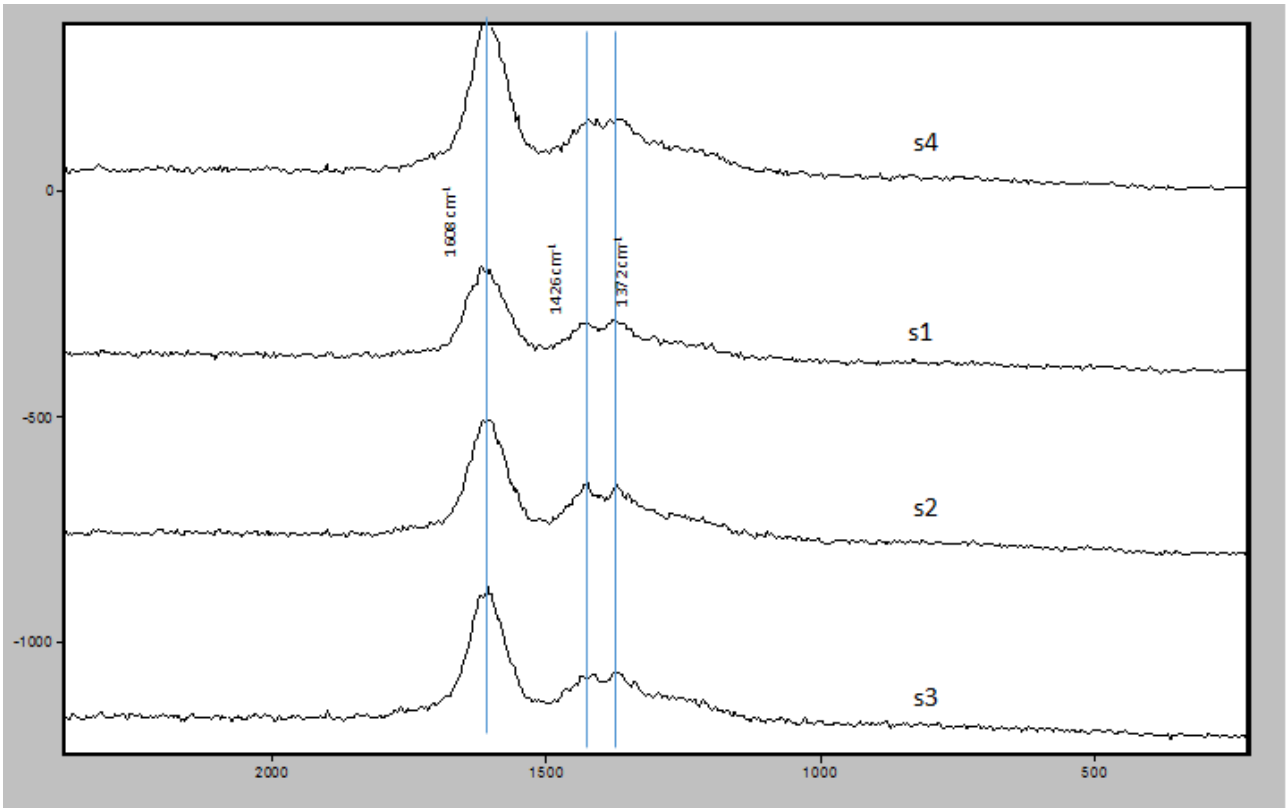

Figure 6-1. UV resonance Raman (UVRR) of extractives from four willow inner bark clones collected at the excitation wavelengths of 244 nm.

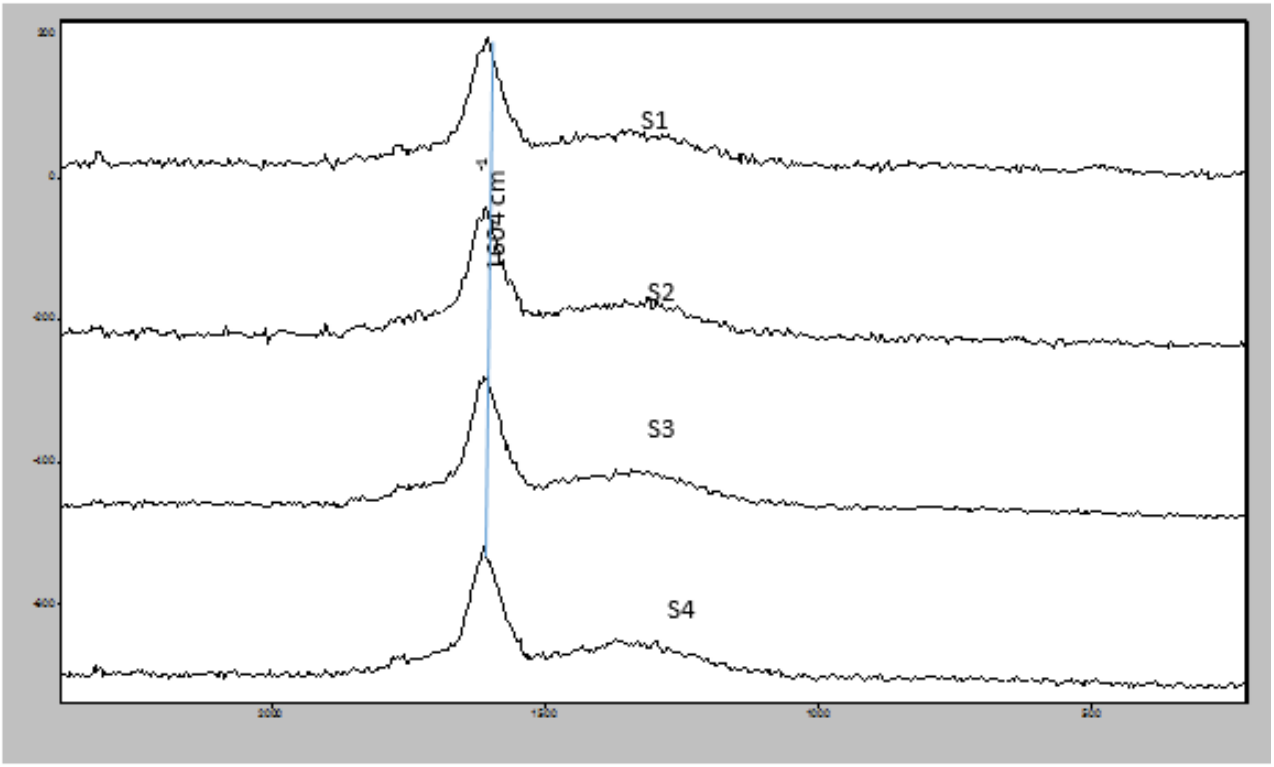

Figure 6-2. UV-Raman of lignin from four willow clones collected at the excitation wavelengths of 244 nm.

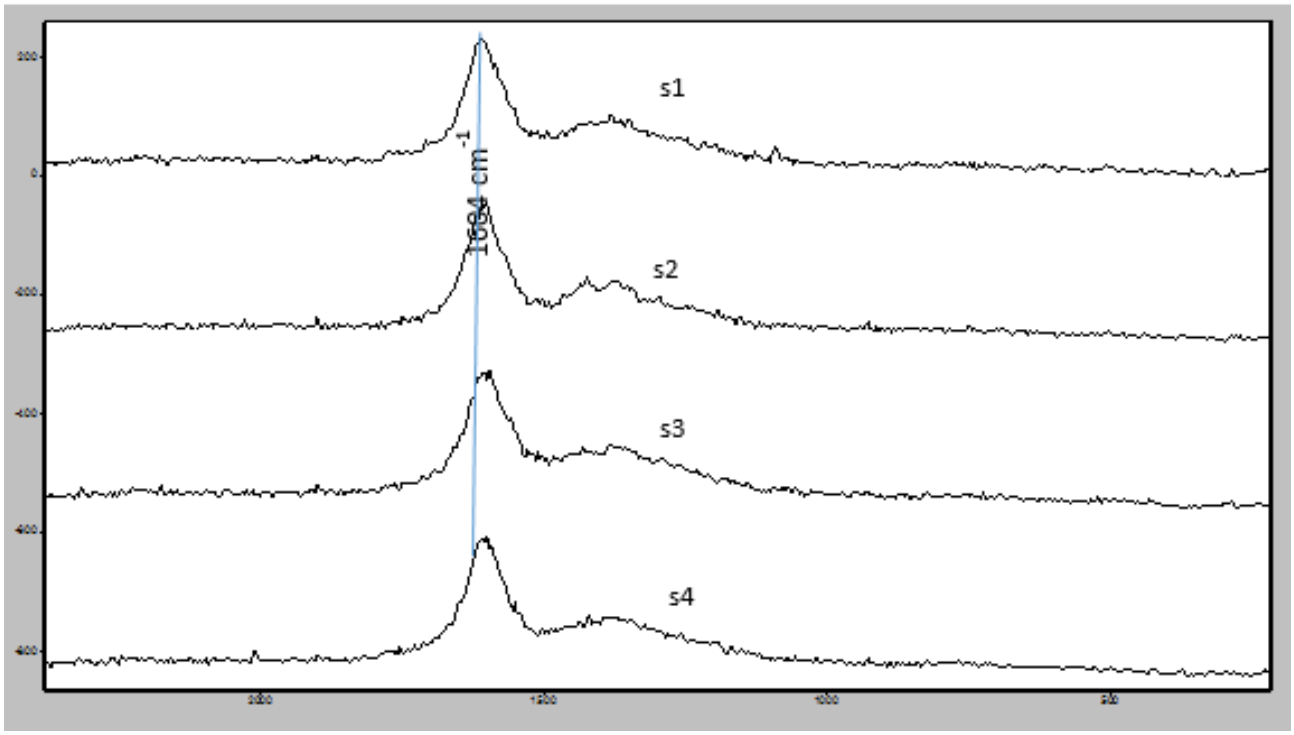

Figure 6-3. UV-Raman of **willow inner bark (inside)** from four willow clones collected at the excitation wavelengths of 244 nm.

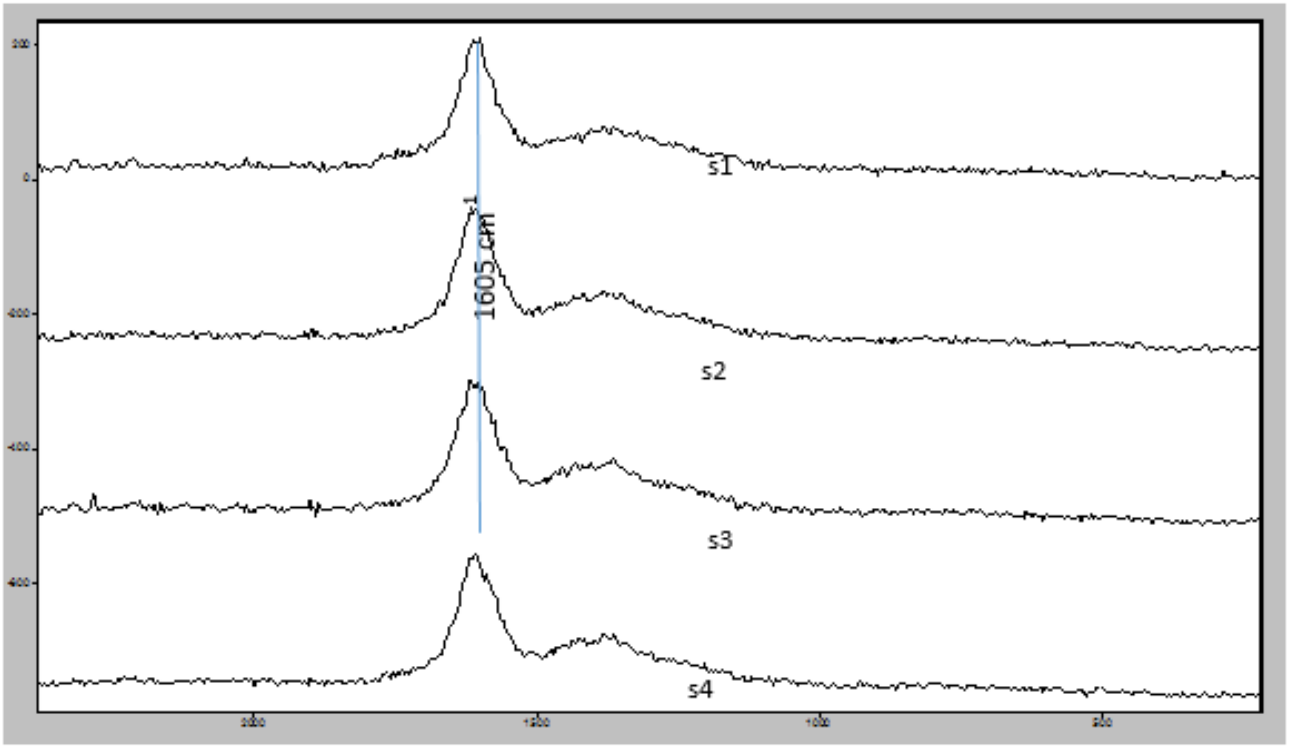

Figure 6-4. UV-Raman of **willow inner bark (outside)** from four willow clones collected at the excitation wavelengths of 244 nm.

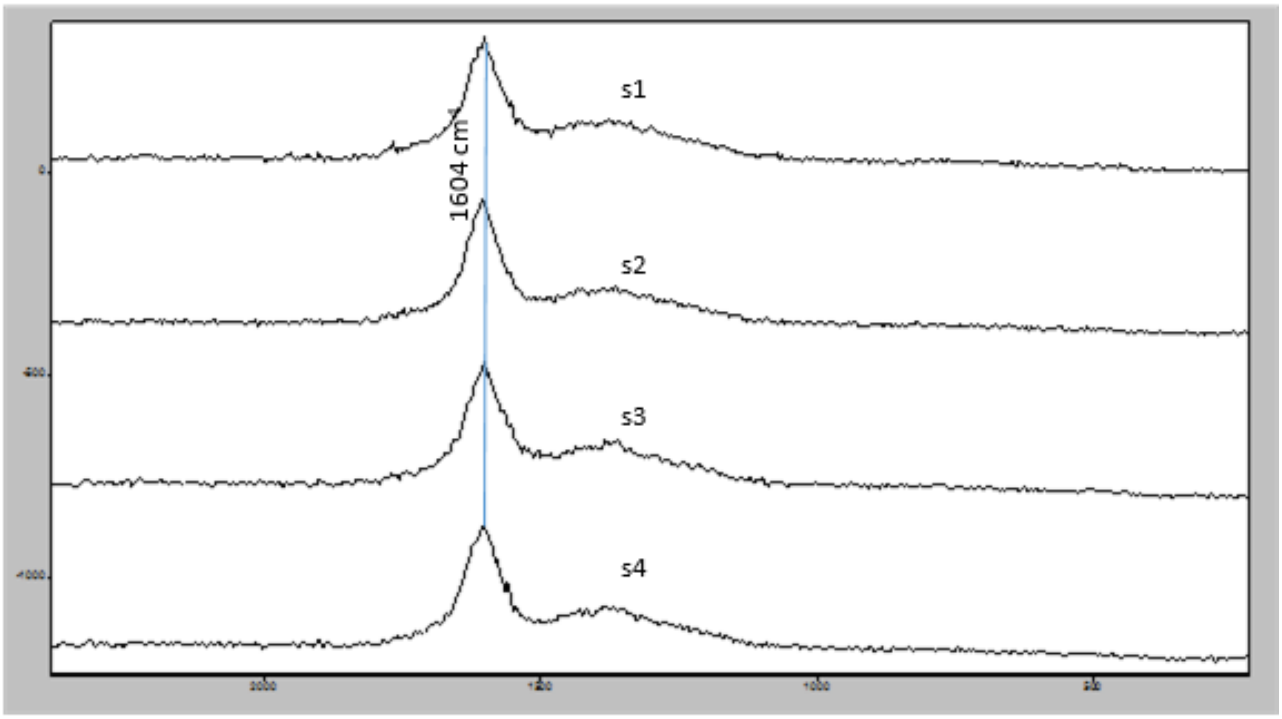

Figure 6-5. UV-Raman of **willow extracted inner bark** after maceration (without lignin) from four willow inner bark clones collected at the excitation wavelengths of 244 nm.

7 Previous study

All materials used for previous study have no official provenance (provided by Professor Vuorinen Tapani)

7.1 Fibre properties

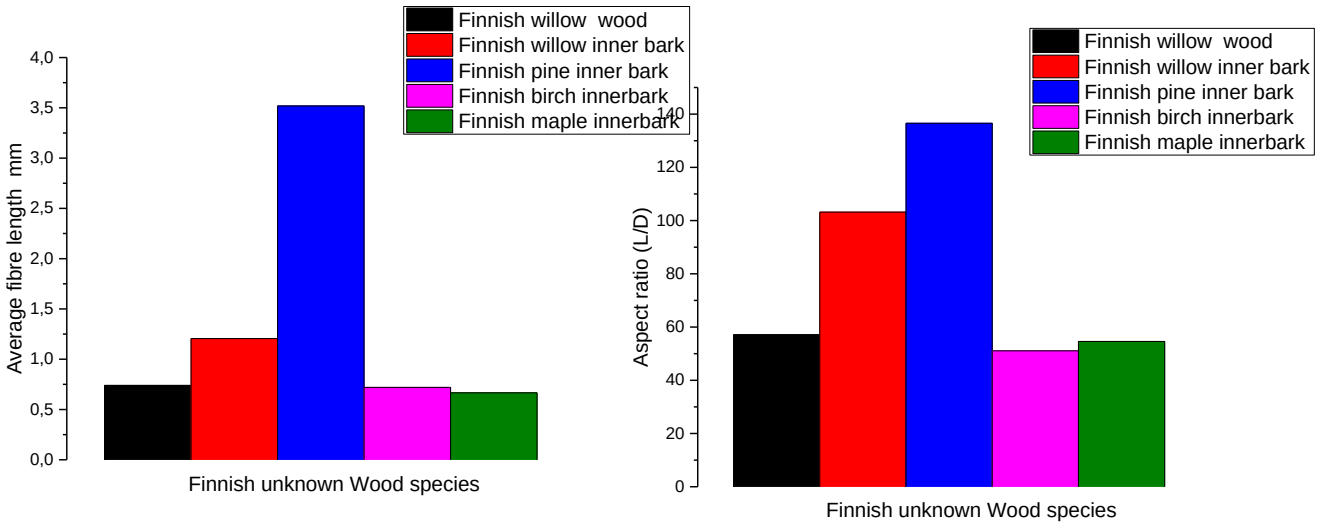

Figure 7-1. Fibre properties of the preliminary studied wood species (willow and pine from Kraft cooking. birch and maple from maceration process).

Table 7-1-1. Specific data for the preliminary fibre studies.

	Average fibre length (mm)	Average fibre width (um)	Aspect ratio (L/D)
Finnish willow wood	0.7	13.0	57.1
Finnish willow inner bark	1.2	11.7	103.2
Finnish Pine inner bark	3.5	25.8	136.6
Finnish Birch innerbark	0.7	14.1	51.1
Finnish Maple innerbark	0.7	12.2	54.6

Table 7-1-2. Specific cooking recipe for kraft cooking (willow/ pine) and cell dissociation (maple/birch).

Method 1 Recipe for the Kraft cooking (Pine and willow)		
	Number	Unit
Oven Dry chip mass	30.0	g
Chip dry matter content	0.9	
Liquor ratio	4.5	
Alkali charge(active alkali as NaOH)	0.2	
Sulphidity	0.4	
NaOH -solution Concentration.active alkali	152.7	g NaOH/l
Na_2S-solution Concentration.active alkali	53.0	g NaOH/l
Amount of chips	33.1	g
Amount of water in chips	3.1	ml
Amount of active alkali	6.9	g
Amount of which sulphidy	2.4	g
Amount of which NaOH	4.5	g
Na_2S-solution	45.6	ml
NaOH -solution	29.4	ml
Water to be added	57.0	ml
Maximum temperature	170.0	^oC
Time to maximum temperature	11.0	min
Time at maximum temperature	120.0	min
Method 2 Maceration for cell dissociation (Maple and Birch)		
1.05 kg/l Acetic acid (96 %)	0.5	ml
1.11 kg/l H_2O_2 (30 %)	0.5	ml
Time	48	Hour
Temperature	40	^oC

7.2 Paper sheet making (property measurement)

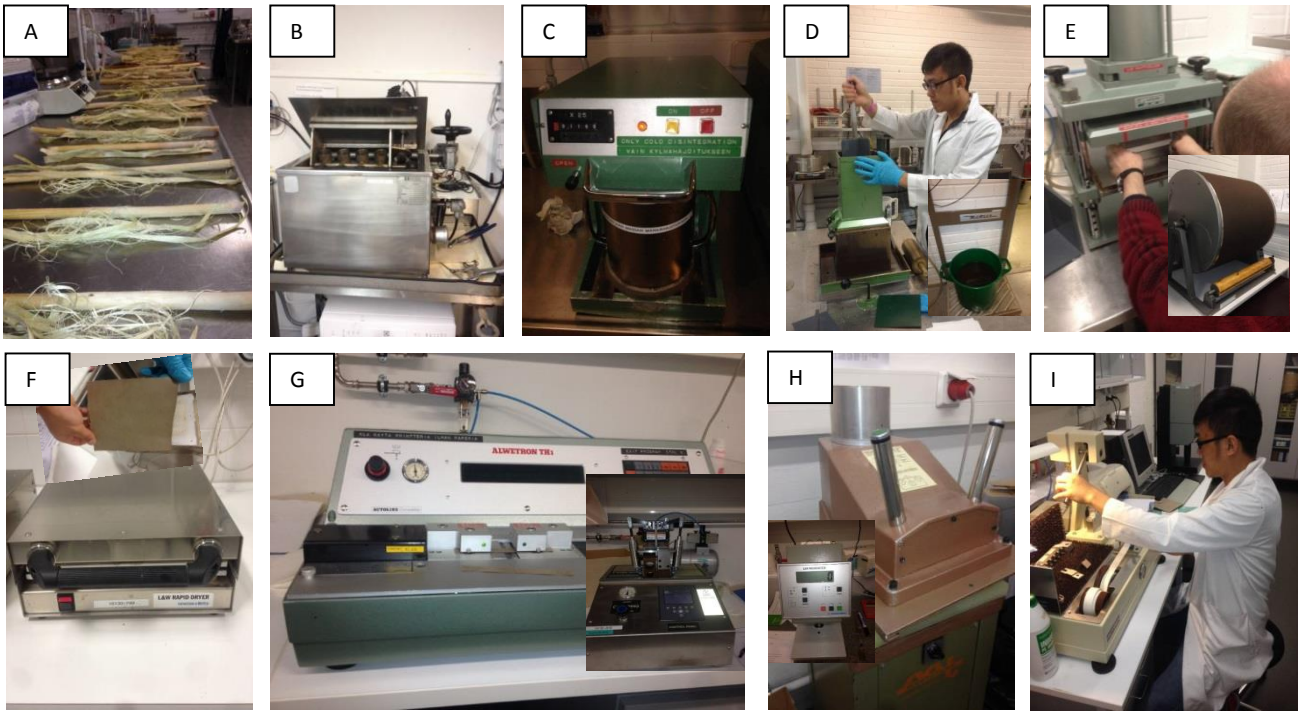

Figure 7-2-1. Process for making inner bark paper sheet and property measurement: (A) Debarking material, (B) Ratio: 5.5 Alkali charge: 0.23 Sulphidity: 0.35 120 min/ 170°C, (C) 30min/ 30000 r, (D) 60 g/m^2 consistency: 2 g/l, (E) Relative humidity: 50% 23 ℃/ 4h, (G) Zero-span testing and tensile strength testing, (H) Cutting into 141mm x 141mm, (I) Internal bond measurement.

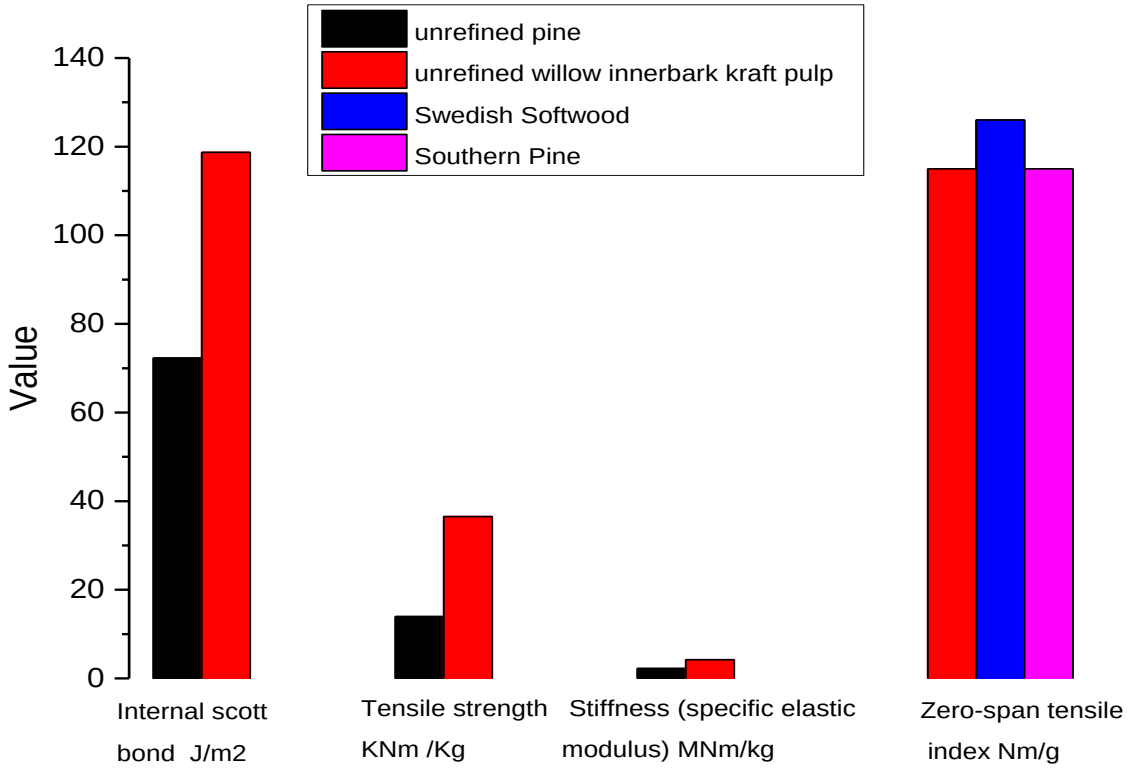

Figure 7-2-2. Comparison for the inner bark sheet properties with other wood species.

Table 7-2. Specific data for the inner bark sheet properties with other wood species.

	Internal scott bond (J/m2)	Tensile strength (KNm/Kg)	Stiffness (specific elastic modulus) (MNm/kg)	Zero-span tensile index (Nm/g)
Unrefined pine [Lauri et al. 2014]	72.28	13.94	2.224	not defined
Unrefined willow innerbark kraft pulp	118.7	36.5	4.209	115
Swedish Softwood [Hanna Karlsson.(2010]	not defined	not defined	not defined	126
Southern Pine [Hanna Karlsson.(2010]	not defined	not defined	not defined	115

7.3 Composite application

Figure 7-3-1. Use of willow inner bark fibre as matrix for polymer composite: (A) Filtration pretreatment (triple with 99.5% ethanol 3H once with 100% acetone 1H) prevent the bonding for causing large aggregates/ particle networks in the dried pulp fibre, (B) Dry in the fume hood, (C) Midi-extruder machine 135 °C 4 min/ 65 rpm, (D) Injection + moulding machine + air machine (Polymer lab. Aalto University), (E) Unbleached Kraft cooking willow + Bleached Pine (60% pine. 40% spruce) PE polymer, (F) Strength measurement.

Table 7-3. Data for the polymer measurement.

	Thickness (um)	Weight (g)	Cross section area (m^2)	Grammage (g/m^2)	Elastic modulus (GPa)	Yield strength (Mpa)	Tensile strength (Mpa)	Max load (N)
Pure PE	1444	0.8320	0.0017	490.37	0.21	1.70	10.21	73.75
Pine 10%	1446	0.8682	0.0017	512.	0.47	1.2	11.14	80.27
Pine 20%	1454	0.9227	0.0017	547.59	0.66	1.57	14.52	105.61
Pine 30%	1451	0.9619	0.0017	569.66	0.80	1.97	16.36	118.74
willow 10%	1447	0.8666	0.0017	511.82	0.31	1.41	11.16	80.79
willow 20%	1431	0.8973	0.0017	524.06	0.61	1.71	14.78	105.78
willow 30%	1476	0.9759	0.0017	587.90	0.73	2.02	16.45	121.42

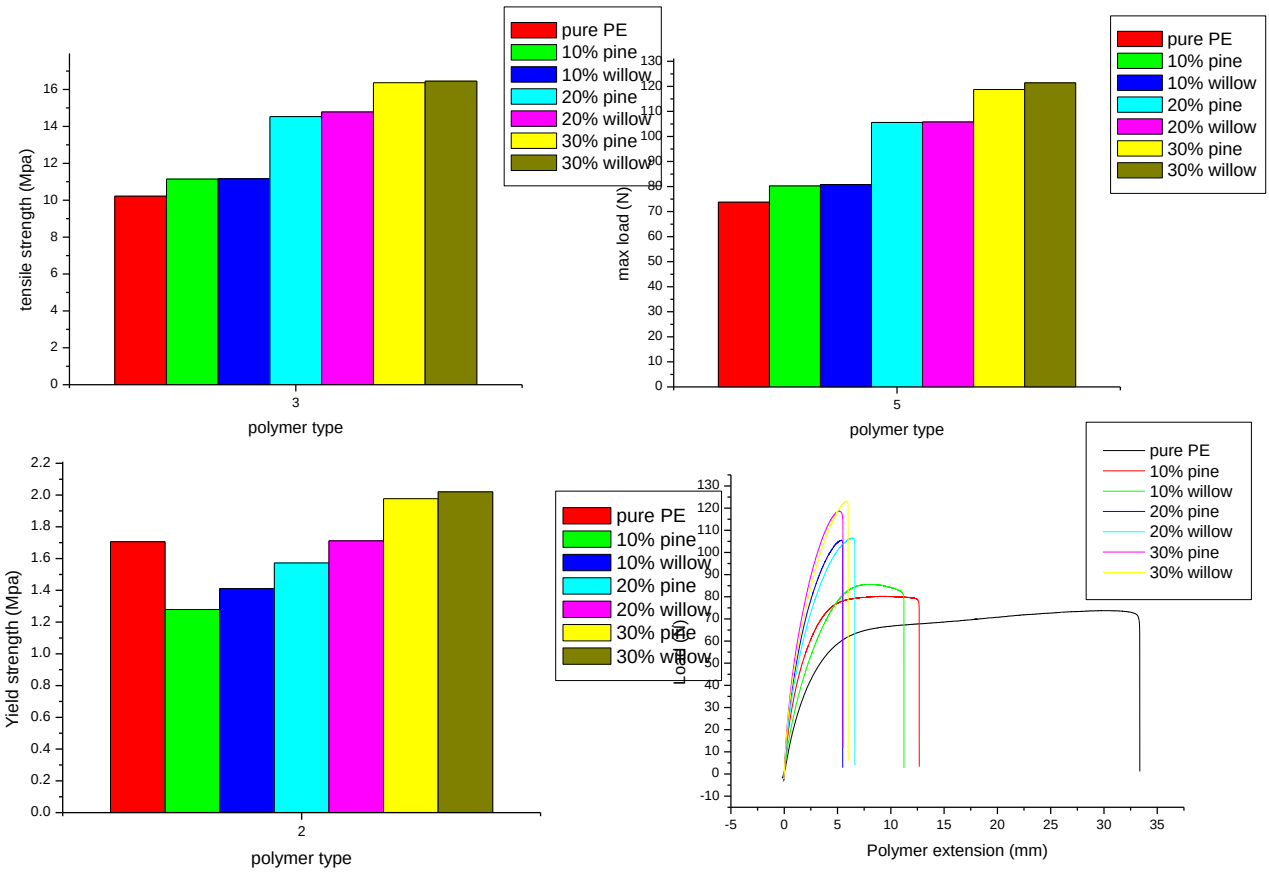

Figure 7-3 -2. Data for the polymer measurement.

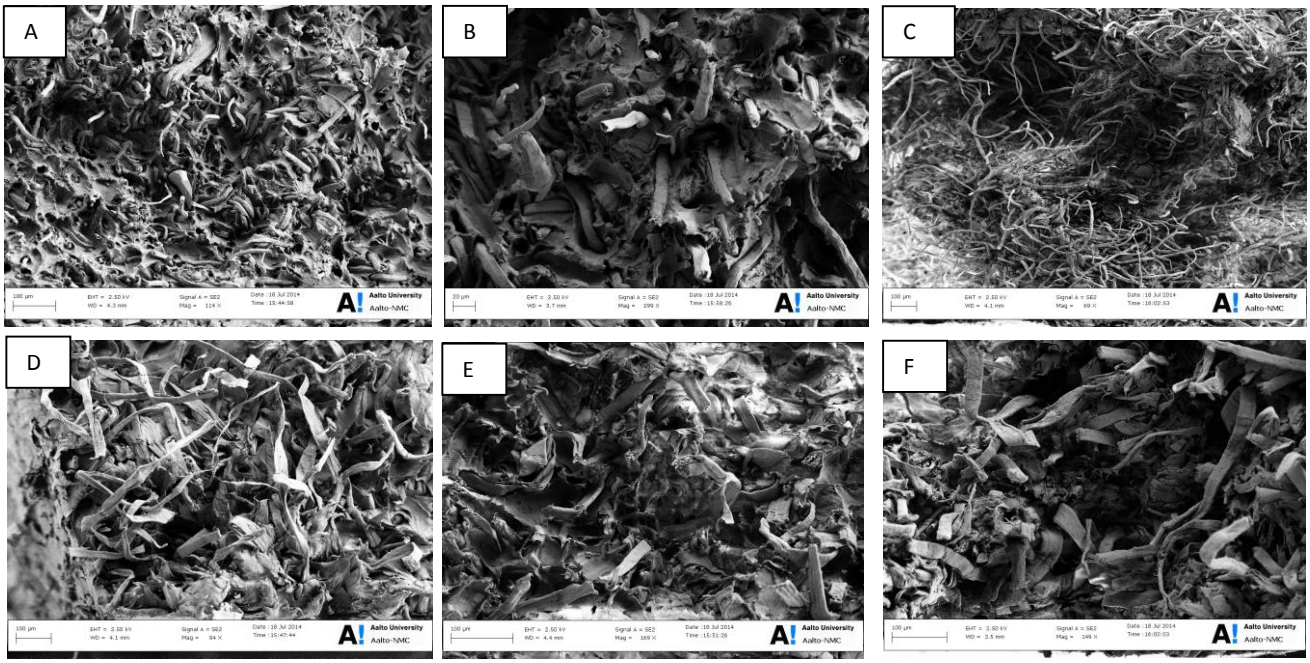

Figure 7-3-3. SEM image for the polymer breaking section, (A) 10% willow, (B) 20% willow, (C)30 % willow, (D) 10% pine, (E) 20% pine, (F) 30% pine.

7.4 Cooking method

Table 7-4-1. Recipe for Non-sulfur alkaline hydrogen peroxide (AHP), Sulphite anthraquinone cooking, Kraft cooking.

Cooking type	Material	T. (℃)	t. (min)	Liquor ratio	Na_2SO_3 % on o.d. bark	AQ % on o.d. bark	Final PH	Tot. Yield (%)	Kappa number	ISO-brightness. (%)	Viscosity. (ml/g)
Sulfite AQ (165 ℃)	willow innerbark	165	240	4	40	0.2	7.9	45.0	85.4	18.1	1157
Sulfite AQ (170 ℃)	willow innerbark	170	240	4	48.5	0.2	7.8	47.9	47.5	18.9	526
Cooking type	Material	T. (℃)	t. (min)	Liquor ratio	Alkali charge (as NaOH) (%)	Sulphidity (%)	Final PH	Tot. Yield (%)	Kappa number	ISO-brightness. (%)	Viscosity. (ml/g)
Kraft cooking (170 ℃)	willow innerbark	170	120	5	23	35	11.72	38	33.5	13.5	1266
Cooking type	Material	T. (℃)	t. (min)	Liquor ratio	Alkali charge (as NaOH) (%)	H_2O_2 % on o.d. bark	Final PH	Tot. Yield (%)	Kappa number	ISO-brightness.(%)	Viscosity. (ml/g)
AHP cooking (165 ℃)	willow innerbark	165	90	5	20	3	11.3	40.6	45.6	7.8	1097
AHP cooking (170 ℃)	willow innerbark	170	120	5	20	6	10.0	44.5	48.9	6.5	654

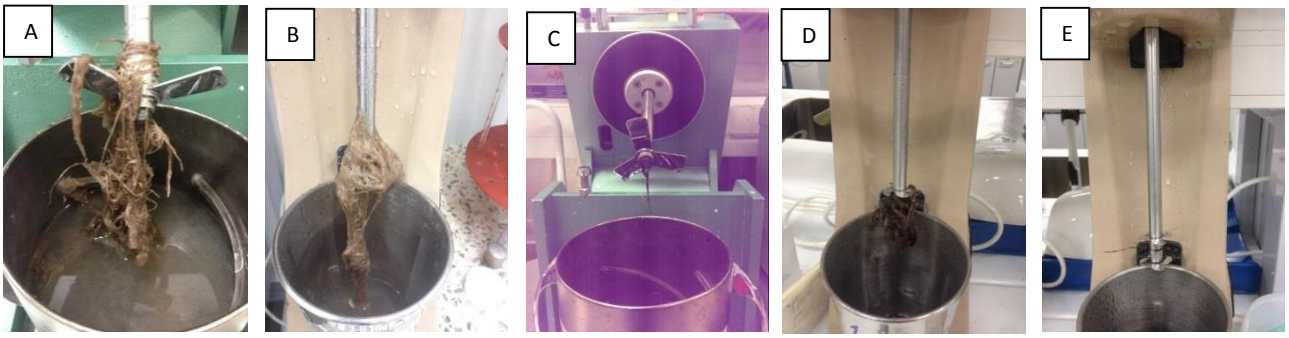

Figure 7-4-1. (A) Sulfite AQ (165 ℃), (B) Sulfite AQ (170 ℃), (C) Kraft cooking (170 ℃), (D) AHP (165 ℃), (E) AHP (170 ℃).

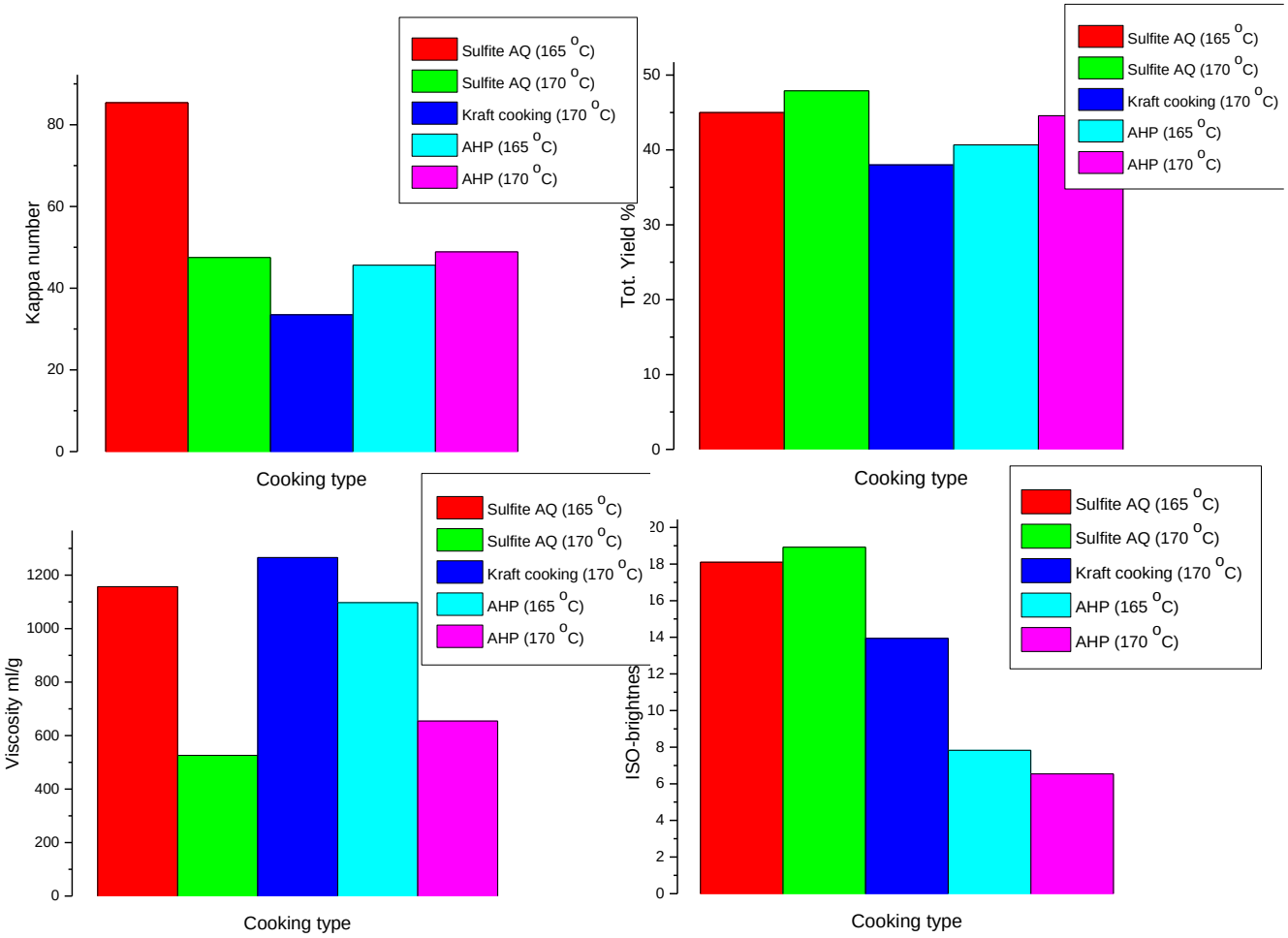

Figure 7-4-2. Comparison of the cooking method (Kappa/Viscosity/ISO/ Yield).

7.5 Infrared Raman spectroscopy of the willow inner bark

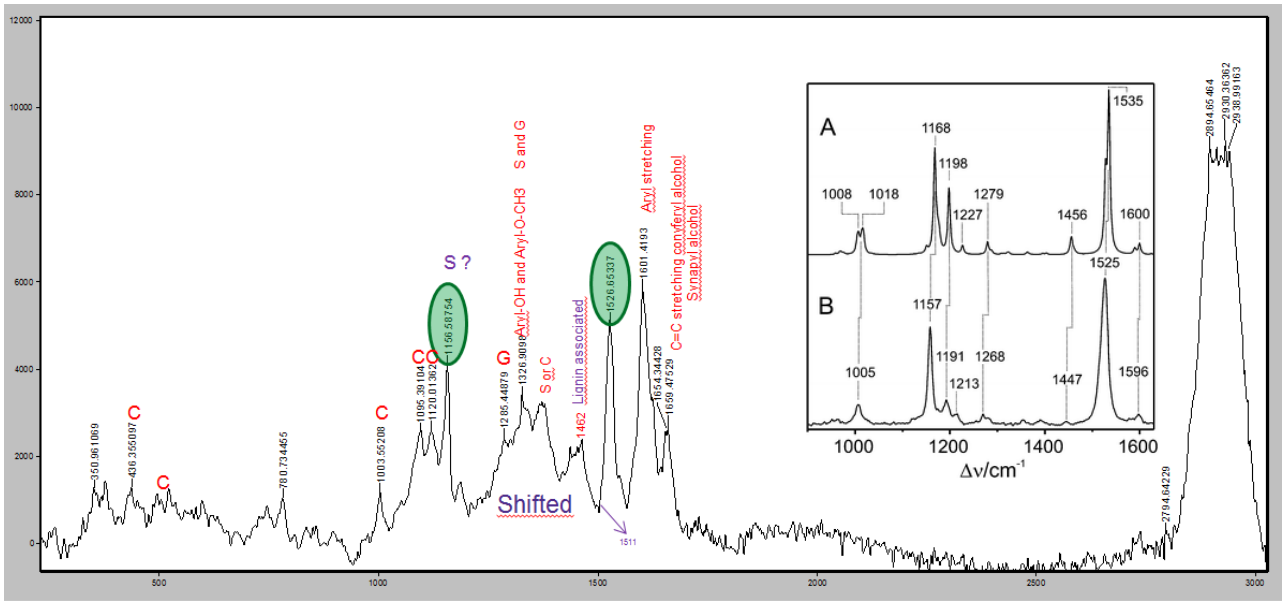

Figure 7-5. Willow inner bark (local native willow) spectra of inner bark without any treatment under near infrared Raman spectroscopy (785nm) and 20 mW, one interesting band named b-carotene with bands that reported at 1005, 1157 and 1525 cm^{-1} spectrum B above in figure 7-5 (Tschirner 2009). The carotene could be so high to cause these intensive signals (Schulz H. 2005).

7.6 Raman spectroscopy

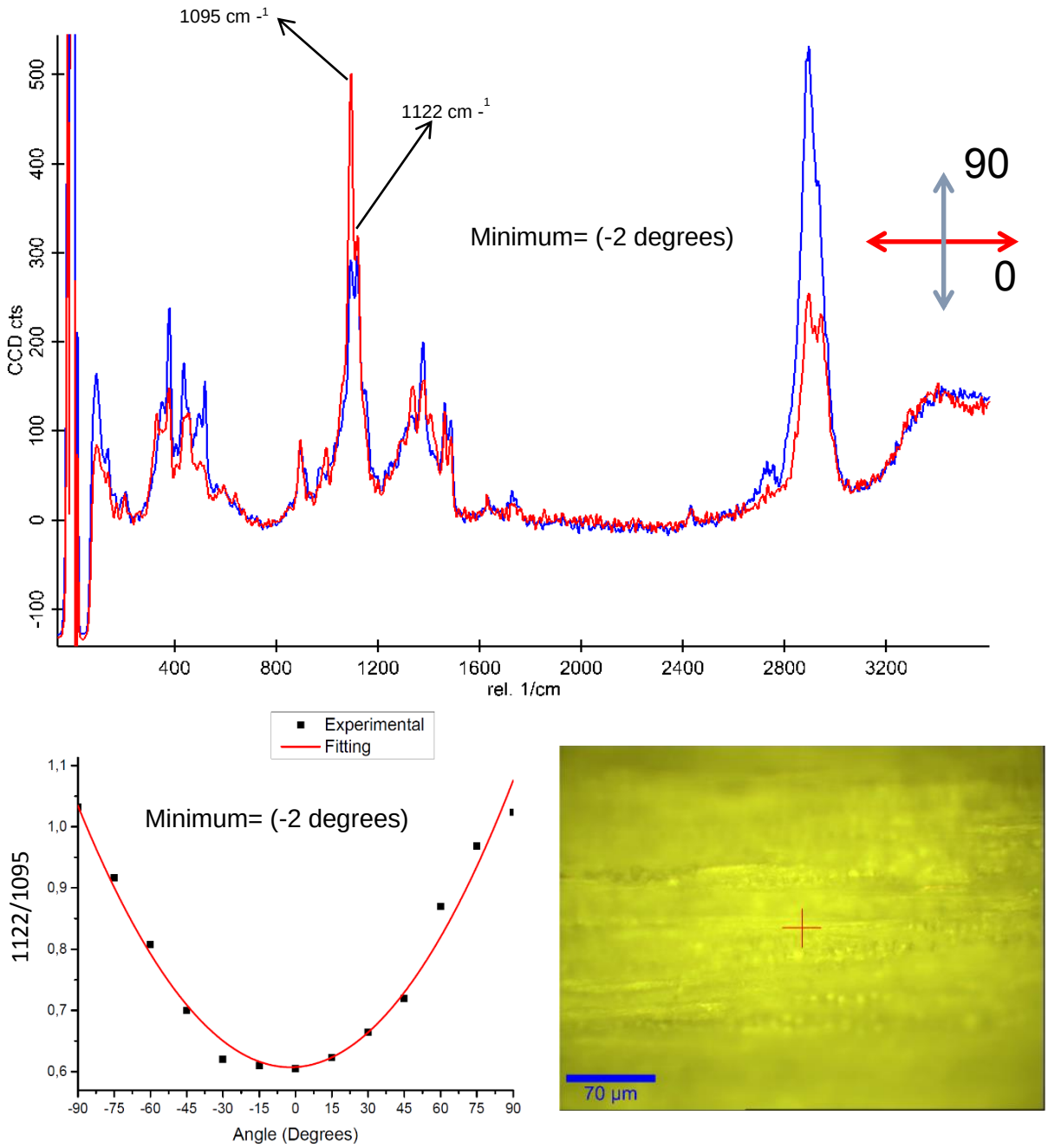

Figure 7-6. Raman mapping for the cellulose fibrial angle.

Figure 7-6 shows the characteristic band for cellulose is apparently shown while lignin is almost removed by maceration (around 1600 cm-^{1}), willow inner bark fibre's micro fibrils are almost align to the axis fibre (~2^{o}), which is comparable to the reference that cellulose fibril orientation is known to be parallel without an angle with respect to the fibre axis (Barnett, Bonham 2004).

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